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Interaction of physicochemical parameters and the blue crab *Portunus pelagicus* (Linnaeus, 1758) in the Arabian Gulf

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Abstract

Heavy metals are not biodegradable and can accumulate in living tissues along the food chain, reaching humans mainly through food. Crabs and other organisms that feed on organic matter in estuarine ecosystem can absorb a greater burden of these toxic elements and thus pose a potential risk to the health of the region's population. Blue crabs collected along three estuaries of the Arabian Gulf. In this work, Measurement of water temperature in ^{o}C , salinity g/l, conductivity in Siemens per meter (S/m), water turbidity in NTU, total hardness g/l, water PH in mg/l and water dissolved oxygen in mg/l were measured by a probe HORIBA® mod. U-22/Water Quality-Checker [1]. Quantitative studies were carried out on Cu, Zn, Cd, Pb, Cr, Al , Fe, Mn and Ni content in sea water and in the gills of the blue crab *Portunus pelagicus* (n = 480). Analysis of heavy metals was performed by energy dispersive x-ray fluorescence (EDXRF). Values of salinity g/l, conductivity in Siemens per meter (S/m), water turbidity in NTU, total hardness g/l and water PH in mg/l are within the recommended range CONAMA Resolution No. 357/2005 European Union standards [2], Saudi Arabian Standards [3], WHO [4]. Collected data show that crabs live in Southern Khobar estuary contain

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percentage of heavy metals ≥ Saudi Arabian Standards whereas crabs live the Northern Khobar and Ad-dammam estuaries contain percentage of heavy metals ≈ Saudi Arabian Standards.

Keywords: Biodegradable, heavy metal, contamination, blue crab, physicochemical parameters, EDXRF.

1. INTRODUCTION

The Arabian Gulf was subjected to three wars in the last three decades, the Iraq–Iran war in 1980–1988 and the first and second Gulf wars in 1991 and 2003 [5-7]. As a result, a massive oil spill in 1991 in which 6–8 million barrels of Kuwait crude oil were released in the Arabian Gulf as well as various spills from normal oil operation and tanker-related spills [8].This oil spill was considered the largest oil spill in the history. Therefore, numerous studies focused on the fate of this spill and provided evidence that the oil spill effect was limited to 400 km from the spillage point to Saudi Arabian coastline and that the main contaminants were rapidly degraded [9-12]. Both urban and industrial activities on the Arabian Gulf have resulted in elevated levels of metals in filter feeding marine crustacean and bivalves [13-15]. Moreover, the pollution status of the Arabian Gulf is generally attributed to the direct discharge of wastes due to the high level of urbanization and industrialization in the environs [9, 16]. Since the later part of the 19^{th} century, the Arabian Gulf has served as the ultimate sink for disposal of untreated domestic sewage. Almost all chemical elements are involved in closed cycles in nature, at concentrations that do not cause harmful effects to organisms, moving between the various environmental compartments at varying speeds and extensions [8, 17]. However, one of the most serious aspects of introducing chemicals into these compartments is their bioaccumulation in the food chain in aquatic and terrestrial environments [18, 19]. The most frequently occurring heavy metal poisonings are caused by aluminum, arsenic, barium, beryllium, cadmium, copper, lead, iron, manganese, mercury, nickel and zinc [15, 20], These elements alter cell structures, enzymes and replace metal cofactors with enzymatic activities [3, 21, 22]. Some heavy metals such as chromium, copper and zinc, found in nature in soil, air and water, in addition to food, are considered to be essential microelements to the metabolism of living organisms [23, 24]. However, the excess or lack of these elements can lead to disorders in the body, and in extreme cases, even death. These essential microelements can be introduced into living tissues through water, food, respiration and even the skin itself. Meantime, according to health organizations, 90% of heavy metals and other contaminants are ingested through food intake [25, 26]. Some heavy metals such as copper, iron, manganese and zinc are essential for vitality of living organisms including man [12, 27-29]. However, these elements show toxic effects when present in higher concentration. Other elements such as lead and cadmium are not essential for metabolic activities and exhibit toxic properties. Lead, cadmium and mercury have no known biological function [30, 31]. Other elements as aluminum, chromium, selenium, silver, arsenic, and antimony have contributed to serious problems in freshwater, estuarine, and coastal ecosystems.

Effects of heavy metals at higher trophic levels include delayed embryonic development, malformation and reduced growth of adult fishes, molluscs and crustaceans [9, 17, 24, 28, 32-34].

In the Arabian Gulf countries, there are important metallurgical, petrochemical, fertilizer and other polluting industries in the region since the 1950s, taking advantage of the facilities of the largest seaport in the estuarine ecosystem [35]. The pollutant material released in the region by the industries, without an adequate emission control program, led to a process of intense environmental degradation, causing destruction on the slopes of coastal line regions, with visible damage to fauna and phanerogamic vegetation. Studies carried out by [9, 13, 36-40] on samples of water, sediments and aquatic organisms from the Arabian Gulf, Norway, southeast Australian waters, found the presence of some heavy metals and organic compounds in concentrations many times above the limits recommended by [3, 18, 41]. However, in the latest report released by [17], the results show a reduction in environmental contamination compared to previous studies for some metals (cadmium, lead, mercury) and some organic compounds (hexachlorobenzenes) [36, 42, 43]. The return of fish and other aquatic organisms to the Arabian Gulf basin does not guarantee the full recovery of the ecosystem, since some chemical compounds, including heavy metals, may reside in the environment, especially in sediments, for long periods of time.

The fishing of crab of genus *Portunus pelagicus* (crustacea, portunidae), also known as blue crab, is an activity of great commercial importance in many parts of Arabian Gulf. In Saudi Arabia, the commercialization of blue crab has been mainly done by the low-income population and by many fishermen who make daily crab fishing their livelihood and the food base of their families. Since most crabs are sold to restaurants and bars on the outskirts, as well as the region's roads and highways, heavy metal analysis is recommended as a way of preventing possible transfer of these pollutants to the general population. The crabs of the family portunidae are common in coastal habitats of tropical, subtropical and temperate regions [30]. The species of the genus *Portunus pelagicus* is widely distributed in the subtropical regions and are very important in the trophic relationships between sandy and muddy bottom fishes and animals [27, 44, 45]. They usually inhabit brackish waters, estuaries and even hypersalines [30, 46, 47]. In Saudi Arabia, crabs are widely distributed on our coast, from Khobar to Dammam estuaries. The main species of blue crabs of the genus *Portunus* sp. that occur in the Arabian Gulf estuaries are*: p. asper, P. convexus, P. elongates, P. iridescens, P. orbicularis and tuberculosus*.

This study aims to measure physical parameters as water temperature in ^{o}C, water conductivity in Siemens per meter (S/m) , water turbidity in NTU, water PH in mg/L and water dissolved oxygen in mg/L and Chlorine. To measure heavy metals and Calcium contaminations of Copper, Zinc, Cadmium, lead, Chromium, Aluminum, Iron, Manganese, Nickel and Calcium in sea water and crab gills (μg/g H_2O , $\mu g g^{-1}$) of the genus *Portunus pelagicus* collected from three estuaries of the Arabian Gulf.

2. MATERIAL AND METHODS

2.1. Sample Collection

The ad-Dammam, Northern and South Khobar estuaries were chosen as the sampling estuaries. The Arabian Gulf is one of the most important industrial effluents receiving bodies of the municipality and the contaminated waters of other coastal estuaries (Metropolitan Region of eastern province). The crabs of genus *Portunus pelagicus* were collected quarterly for one year, from 2017 to 2018 using ring and pyramid capture traps. About 40 crabs, regardless of sex were captured at 30 points about 300 m apart, within a distance of about 10 km along the study estuaries in each season. Since crabs migrate according to tide and salinity, sampling was performed at various locations without concern for the exact location. The captured animals were placed in Styrofoam boxes for transport to the laboratory where they were stored in plastic bags, in a freezer at a temperature of –20 °C for further treatment and analysis.

2.2. Water quality

For analysis of metals in water, Van Dorn's bottles were used for water collection then the samples were packed on ice and transported to the laboratory. Filtration of water samples were preceded in a Millipore type filter ME 25/21 (0.45) using a vacuum pump. 100 ml taken from each sample, 20 ml of P.A. nitric acid was added and 60 ml of solution from each sample was heated. After reaching room temperature, they were 40 ml of each water sample (HNO_3 and HCl in 1: 3 ratio by volume were added) in order to achieve a final solution of 100 ml of sample. To evaluate water quality following physical and chemical parameters Measurement of water temperature in oC , salinity g/l, conductivity in Siemens per meter (S/m), water turbidity in NTU, total hardness g/l, water PH in mg/l and water dissolved oxygen in mg/l, all with the aid of a probe (*HORIBA*® mod. U-22/Water Quality-Checker) [1], calibrated prior to each collection. Readings were made at a depth of 1.0 m. All glass wares used in this work were previously immersed for 24 hours in 2% HNO_3. The goal of this wash was to extract metallic impurities that might be adsorbed on the wall of the containers and could interfere with the analysis of the samples.

2.3. Heavy metals analysis in crab gills

Crabs sampled after thawing at room temperature were identified to species level using a specific identification [48, 49]. Then the crab carapace was removed and the gills were isolated and were weighed to obtain the total fresh (wet) weight. The gills was homogenized and dried in an oven at 150 °C for a minimum of one hour or until a constant weight was obtained.

The methodology used in this work for sample preparation is similar to that recommended by [6]. In summary, masses of about 0.5 to 2.0 g of dry material (according to crab size) were weighed on a force gauge (0.0001 g) digital scale. The sample was placed in a 50 ml beaker, plus 5 ml of concentrated nitric acid per gram of

material used, and the PVC-capped set remained at room temperature for a minimum of 24 hours. After this period, the sample was transferred to a digester block, equipped with a reflux condenser, starting the process at 50 °C, and slowly raising the temperature to 125 °C, remaining until almost complete drying. The remaining liquid residue was then filtered through filter paper, transferred to a 25 ml volumetric flask, and the volume completed with 2% nitric acid solution. After hot acid dissolution, the samples were analyzed for the heavy metal content under study using energy dispersive x-ray fluorescence (EDXRF). High-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) was employed to evaluate the spectral interference in the determination of copper [37]. The standard samples used for spectrophotometer calibration were produced from stock solutions supplied by Tec-Lab with concentrations of the order of 1000 (± 0.3%) ppm. Whenever possible, individual samples of crabs gills were read on the spectrometer in triplicate, and the result for each specimen was obtained by the average of the analyzer. The total uncertainty for the results in these cases was determined by the quadrature sum, taking into account the following partial sources of error: mean standard deviation (4.5-20%), weighing error (0, 04%), volumetric dilution (1%) and spectrometer calibration (0.3%). When it was not possible to prepare two or more samples for the same specimen, the total uncertainty for the measurements was obtained considering the reproducibility error of the method of 10.9% in addition to the other partial sources listed above. This study measured Chlorine, copper, Zinc, Cadmium, lead, Chromium, Aluminum, Iron, Manganese, Nickel and Calcium (μg/g H_2O , μgg^{-1}) in estuarine water and crab gills. Results were calculated and subjected to One-way analysis of variance. Ethical clearance for this study was obtained from Imam Abdulrahman Bin Faisal University ethics committee.

3. RESULTS

3.1. Temperature

The water temperature values are given in (Figure 1a & Table 1a) for the three pan estuaries during the four seasons. Temperature was of Ad-dammam water ≈ North Khobar water ≈ South Khobar water. The mean values of water temperature in Arabian Gulf were 23.17 ± 0.63, 29.17 ±1.19, 17.83 ± 0,55,15.16 ± 0,35°C in spring – winter respectively. One-way analysis of variance (ANOVA) did not show means Signif. Difference at $P < 0.05$ among the three estuaries. Tukey's Multiple Comparison Test showed Mean Dif between Northern Khobar vs Southern Khobar 1.75 at $P < 0.05$ and 3.75 at $P < 0.05$ between Ad-dammam vs Southern Khobar. The lowest 95% CI of diff was recorded -10.69 to 14.19 at $P > 0.05$ between Northern Khobar vs Southern Khobar. The highest 95% CI of diff was recorded -8.694 to 16.19 at $P < 0.05$ between Ad-dammam vs Southern Khobar.

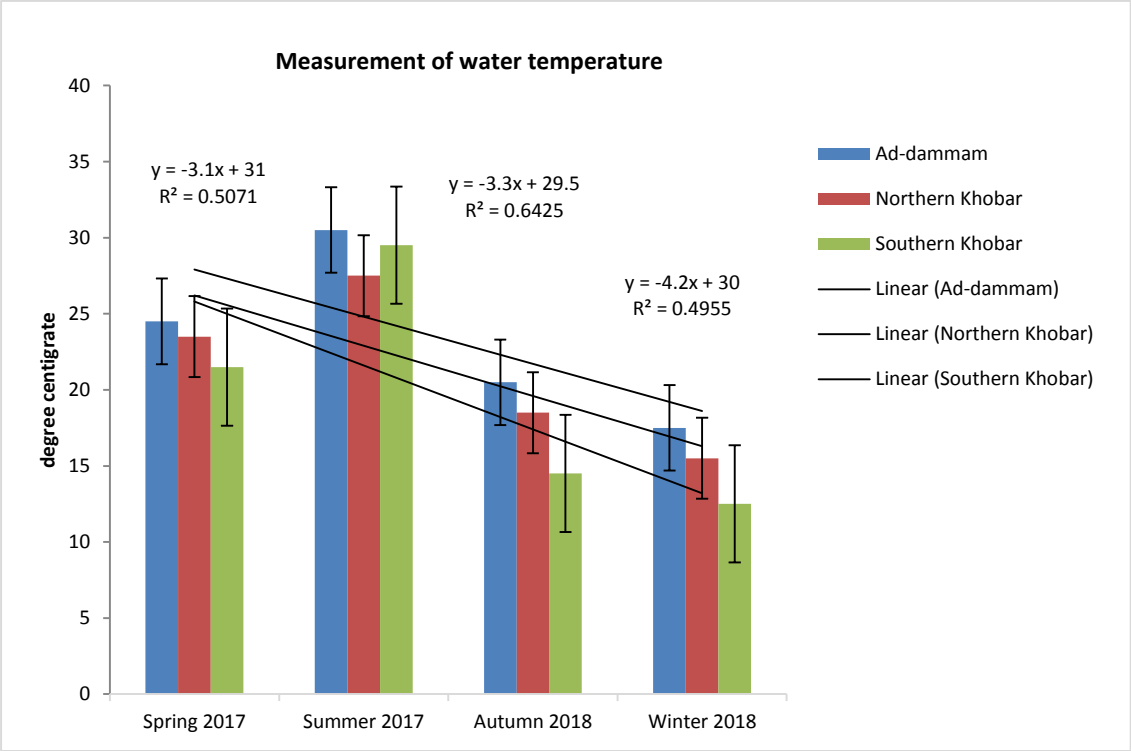

Figure 1a

Table Analyzed 1a				
water temperature				
One-way analysis of variance				
P value	0,7109			
P value summary	ns			
Are means Signif. Diff.? (P < 0.05)	No			
Number of groups	3			
F	0,3545			
R squared	0,07303			
ANOVA Table	SS	df	MS	
Treatment (between columns)	28,17	2	14,08	
Residual (within columns)	357,5	9	39,72	
Total	385,7	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	2	0,6347	P > 0.05	-10.44 to 14.44
Ad-dammam vs Southern Khobar	3,75	1,19	P > 0.05	-8.694 to 16.19
Northern Khobar vs Southern Khobar	1,75	0,5553	P > 0.05	-10.69 to 14.19

3.2. Salinity

The Salinity values are given in (Figure 1b & Table 1b) for the three pan estuaries during the four seasons. Salinity of ad-Dammam water ranged from 3.8 ± 1.02 to 4.9 ± 0.26 g/l, N Khobar 5.7± 0.46 to 5,9 ± 1.3 and S Khobar 6.1± 2.1 to 7,9 ± 0.34 . Salinity was of Ad-dammam water < Northern Khobar water < Southern Khobar water in the four seasons. ANOVA test showed Signif. Difference at $P < 0.05$ for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. -1,75 at $P < 0.01$ between Ad-dammam vs Northern Khobar, -3 at $P > 0.001$ between ad-dammam vs Southern Khobar and -1,25 at $P < 0.05$ between Northern Khobar vs Southern Khobar. The lowest 95% CI of diff was recorded -4.249 to -1.751 at $P > 0.001$ between ad-dammam vs Southern Khobar. The highest 95% CI of diff was recorded -2.999 to -0.5008 at $P < 0.01$ between ad-dammam vs Northern Khobar.

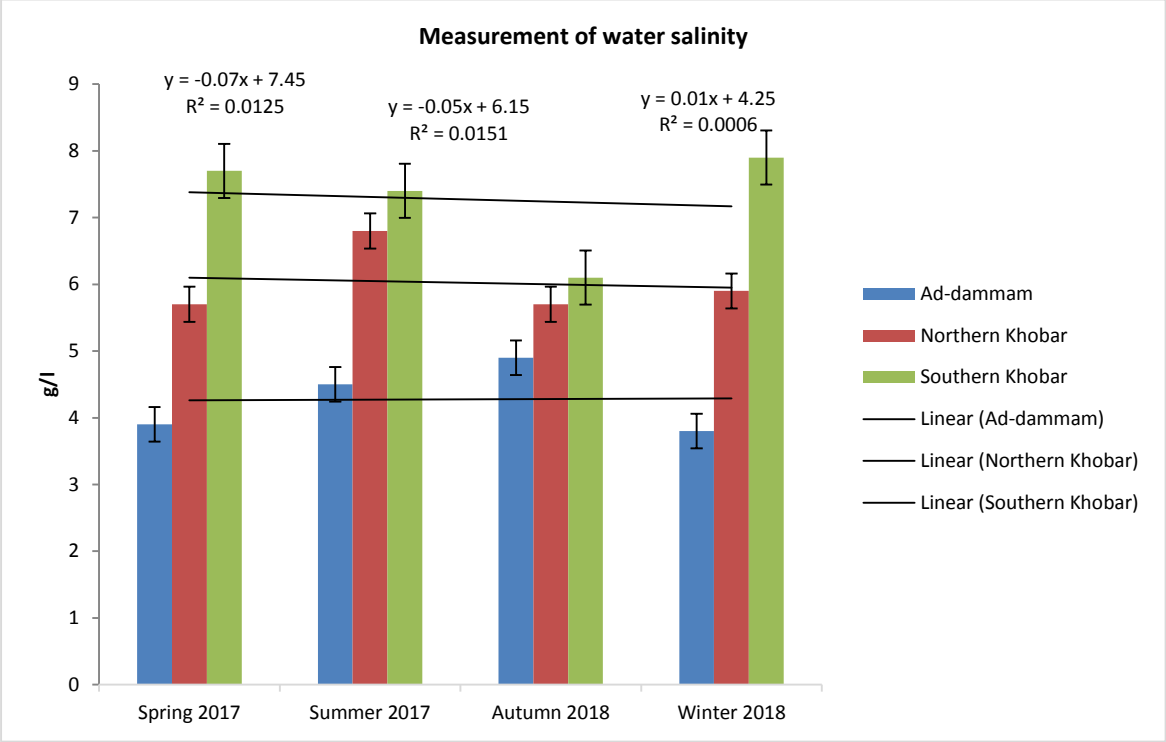

Figure 1b

Table Analyzed 1b				
water salinity				
One-way analysis of variance				
P value	0,0003			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	3			
F	22,69			
R squared	0,8345			
ANOVA Table	SS	df	MS	
Treatment (between columns)	18,17	2	9,083	
Residual (within columns)	3,603	9	0,4003	
Total	21,77	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	-1,75	5,532	P < 0.01	-2.999 to -0.5008
Ad-dammam vs Southern Khobar	-3	9,484	P < 0.001	-4.249 to -1.751
Northern Khobar vs Southern Khobar	-1,25	3,951	P < 0.05	-2.499 to -0.0007829

3.3. Water conductivity

The water conductivity values are given in (Figure 1c & Table 1c) for the three pan estuaries during the four seasons. Water conductivity of ad-Dammam water ranged from 13,12 ±.04 to 19,12 ± 1.13 g/l, N Khobar 10,25 ± 0.24 to 18,1 ± 0.5 and S Khobar 9,12 ± 1.01 to 14,23 ± 0.21. Water conductivity of Ad-dammam water ≈ that of Northern Khobar water > that of Southern Khobar water in the four seasons. ANOVA test did not show Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. 1,395 at P < 0.01 between Ad-dammam vs Northern Khobar, 4,445 at P > 0. 05 between ad-dammam vs Southern Khobar and 3,05 at P < 0.05 between Northern Khobar vs Southern Khobar. The lowest 95% CI of diff was recorded -4.228 to 7.018 at P > 0.001 between Ad-dammam vs Northern Khobar. The highest 95% CI of diff was recorded -1.178 to 10.07 at P < 0.05 between Ad-dammam vs Southern Khobar.

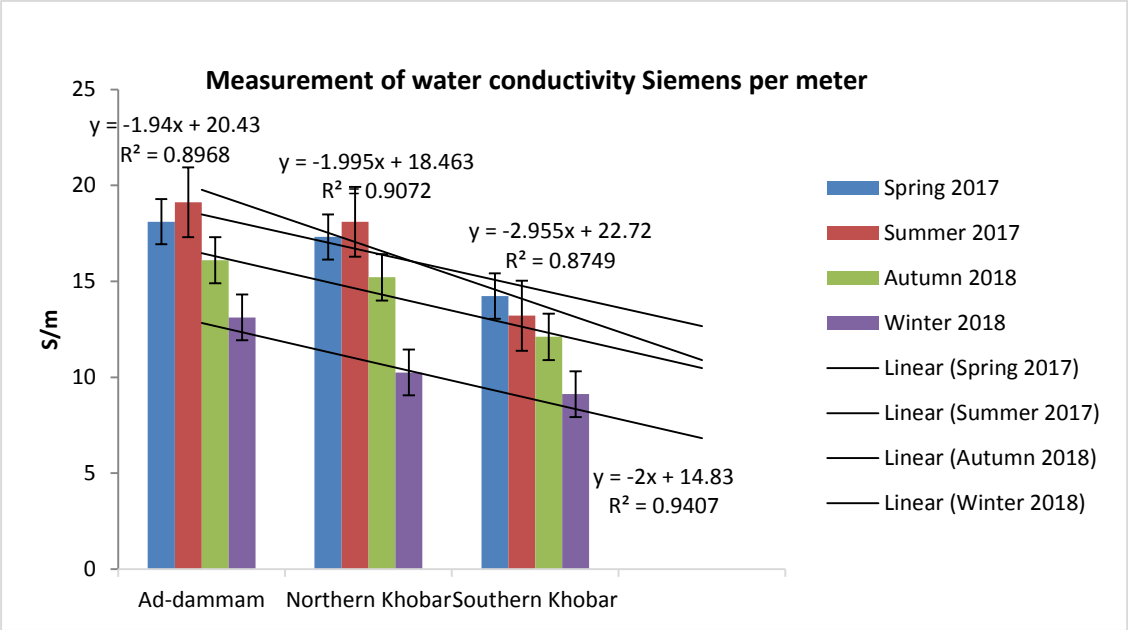

Figure 1c

Table Analyzed 1c				
water conductivity				
One-way analysis of variance				
P value	0,1327			
P value summary	ns			
Are means Signif. Diff.? (P < 0.05)	No			
Number of groups	3			
F	2,549			
R squared	0,3616			
ANOVA Table	SS	df	MS	
Treatment (between columns)	41,34	2	20,67	
Residual (within columns)	72,99	9	8,109	
Total	114,3	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	1,395	0,9797	P > 0.05	-4.228 to 7.018
Ad-dammam vs Southern Khobar	4,445	3,122	P > 0.05	-1.178 to 10.07
Northern Khobar vs Southern Khobar	3,05	2,142	P > 0.05	-2.573 to 8.673

3.4. Water turbidity

The water turbidity values are given in (Figure 1d & Table 1d) for the three pan estuaries during the four seasons. Water turbidity of ad-Dammam water ranged from 7,4 ±.02 to 10,5 ± 0.42 g/l, N Khobar 7.1 ± 0.10 to 11,2 ± 0.5 and S Khobar 13.2 ± 0.51 to 20,2 ± 0.37. Water turbidity of Ad-dammam water ≈ that of Northern Khobar water > that of Southern Khobar water in the four seasons. ANOVA test showed Signif. Difference at $P < 0.05$ for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. -7,525 at $P < 0.01$ between Northern Khobar vs Southern Khobar, 0,025 at $P > 0.05$ between Ad-dammam vs Northern Khobar and -7,5 at $P < 0.01$ between Ad-dammam vs Southern Khobar. The lowest 95% CI of diff was recorded -11.71 to -3.338 at $P < 0.01$ between Northern Khobar vs Southern Khobar. The highest 95% CI of diff was recorded -4.162 to 4.212 at $P > 0.05$ between Ad-dammam vs Northern Khobar.

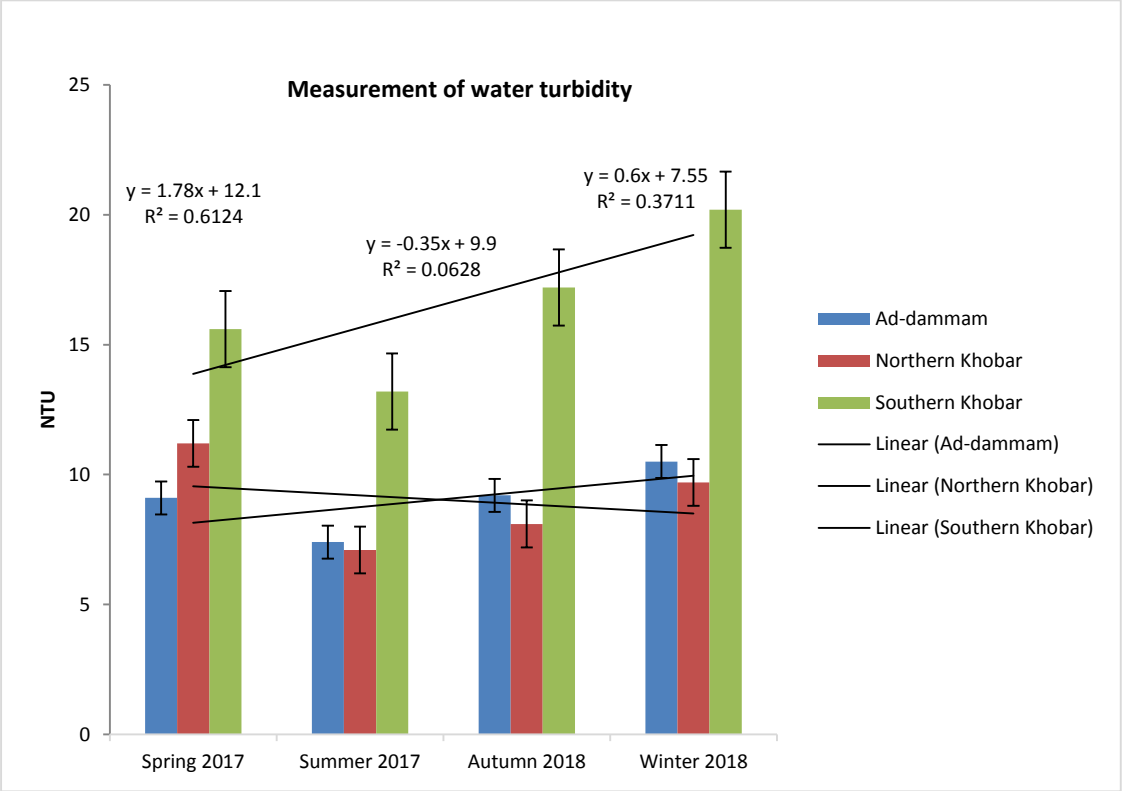

Figure 1d

Table Analyzed 1d				
water turbidity				
One-way analysis of variance				
P value	0,0009			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	3			
F	16,74			
R squared	0,7881			
ANOVA Table	SS	df	MS	
Treatment (between columns)	150,5	2	75,25	
Residual (within columns)	40,47	9	4,496	
Total	191	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	0,025	0,02358	P > 0.05	-4.162 to 4.212
Ad-dammam vs Southern Khobar	-7,5	7,074	P < 0.01	-11.69 to -3.313
Northern Khobar vs Southern Khobar	-7,525	7,097	P < 0.01	-11.71 to -3.338

3.5 Water total hardness

The water total hardness values are given in (Figure 1e & Table 1e) for the three pan estuaries during the four seasons. Water total hardness of ad-Dammam water ranged from 430 ± 1. 2 to 460 ± 2.32 g/l, N Khobar 930 ± 2.20 to 990 ± 2.5 and S Khobar 590 ± 3.51 to 680 ± 2.27. Water total hardness of Ad-dammam water << that of Northern Khobar water < that of Southern Khobar water in the four seasons. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. -527,5 at P < 0.001 between Ad-dammam vs Northern Khobar, 320 at P > 0. 001 between Northern Khobar vs Southern Khobar and -207,5 at P < 0.001 between Ad-dammam vs Southern Khobar. The lowest 95% CI of diff was recorded -584.7 to -470.3 at P < 0.01 between Ad-dammam vs Northern Khobar. The highest 95% CI of diff was recorded 262.8 to 377.2 at P > 0.001 between Northern Khobar vs Southern Khobar.

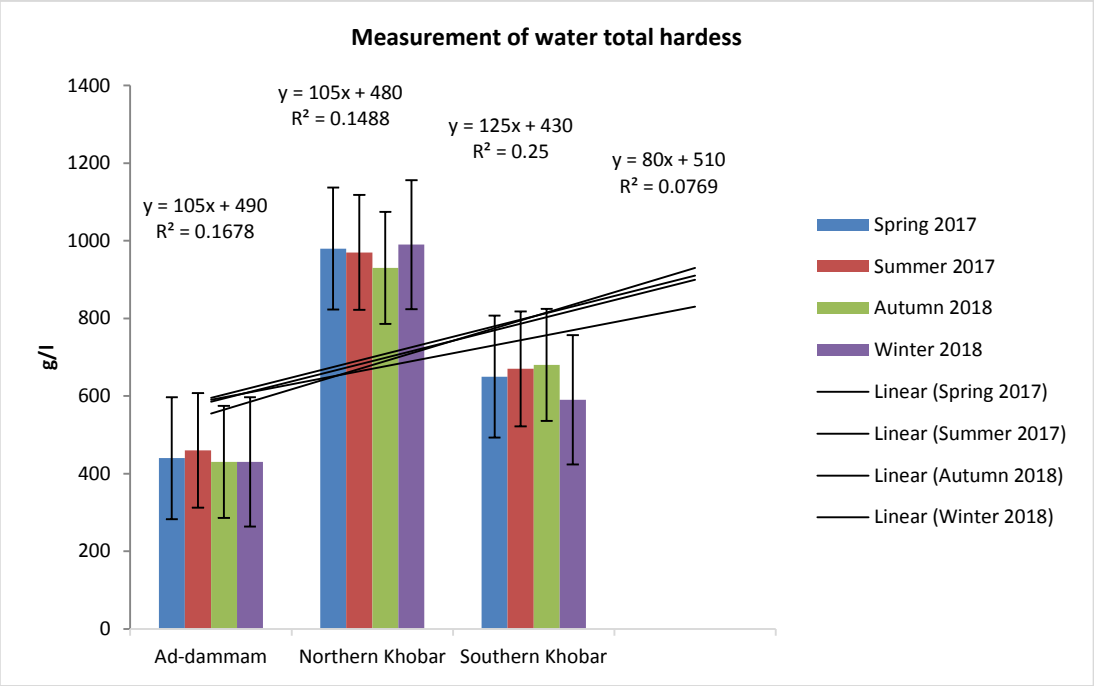

Figure 1e

Table Analyzed 1e				
water total hardness				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	3			
F	336,7			
R squared	0,9868			
ANOVA Table	SS	df	MS	
Treatment (between columns)	565000	2	282500	
Residual (within columns)	7550	9	838,9	
Total	572500	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	-527,5	36,43	P < 0.001	-584.7 to -470.3
Ad-dammam vs Southern Khobar	-207,5	14,33	P < 0.001	-264.7 to -150.3
Northern Khobar vs Southern Khobar	320	22,1	P < 0.001	262.8 to 377.2

3.6. pH concentration

The pH (mg/l) values are given in (Figure 1f & Table 1f) for the three pan estuaries during the four seasons. PH value of ad-Dammam water ranged from 6.2 ± 0.1 to 6.9 ± 1. 2 g/l, N Khobar 6.3 ± 1.10 to 990 ± 0.9 and S Khobar 8.3 ± 1.41 to 10.4 ± 3.14. ANOVA test showed Signif. Difference at $P < 0.05$ for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. -0,65 at $P < 0.05$ between Ad-dammam vs Northern Khobar, -2,2 at $P > 0.01$ between Northern Khobar vs Southern Khobar and -2,85 at $P < 0.001$ between Ad-dammam vs Southern Khobar. The lowest 95% CI of diff was recorded -4.175 to -1.525 at $P > 0.001$ between Ad-dammam vs Southern Khobar. The highest 95% CI of diff was recorded -1.975 to 0.6745 at $P < 0.05$ between Ad-dammam vs Northern Khobar.

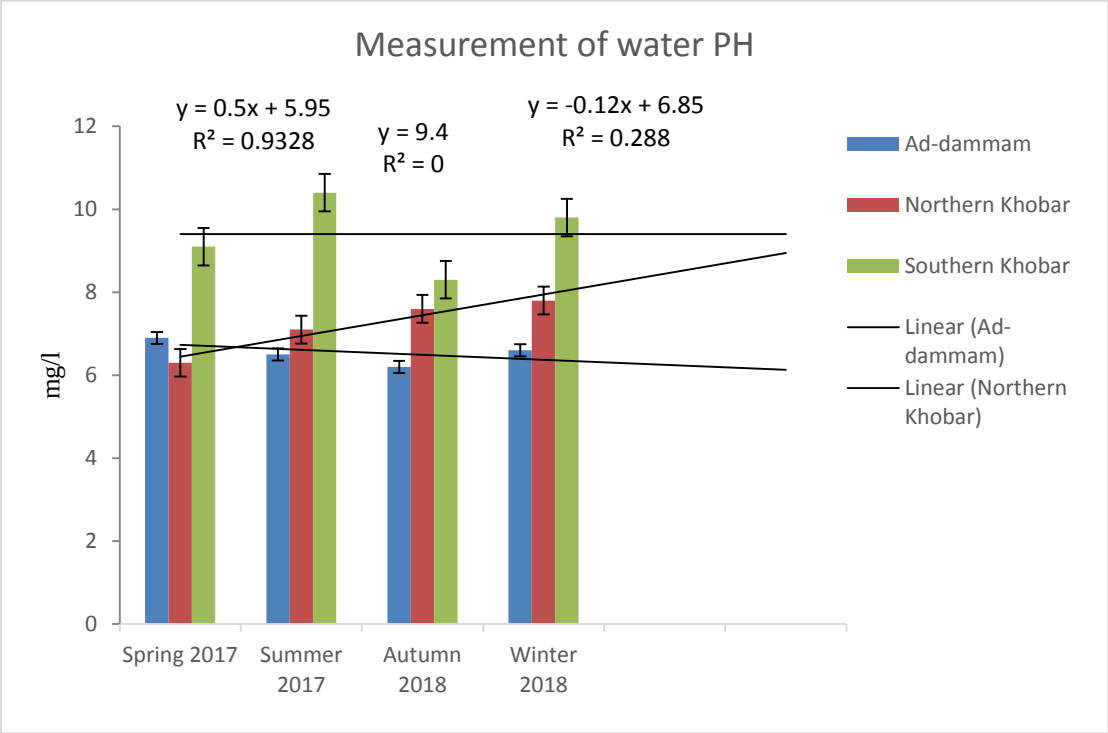

Figure 1f

Table Analyzed 1f				
water PH				
One-way analysis of variance				
P value	0,0005			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	3			
F	19,83			
R squared	0,815			
ANOVA Table	SS	df	MS	
Treatment (between columns)	17,85	2	8,923	
Residual (within columns)	4,05	9	0,45	
Total	21,9	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	-0,65	1,938	P > 0.05	-1.975 to 0.6745
Ad-dammam vs Southern Khobar	-2,85	8,497	P < 0.001	-4.175 to -1.525
Northern Khobar vs Southern Khobar	-2,2	6,559	P < 0.01	-3.525 to -0.8755

3.7. Dissolved oxygen

The dissolved oxygen values are given in (Figure 1g & Table 1g) for the three pan estuaries during the four seasons. Dissolved oxygen value of ad-Dammam water ranged from 8,8 ± 1.1 to 14.1 ± 1. 7 g/l, N Khobar 7.3 ± 1.30 to 8.3 ± 1.70 and S Khobar 4.8 ± 1.21 to 6.3 ± 2.5. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. 3,325 at P < 0.05 between Ad-dammam vs Northern Khobar, 6,5 at P > 0. 001 between Ad-dammam vs Southern Khobar and 3,175 at P < 0.05 between Northern Khobar vs Southern Khobar. The lowest 95% CI of diff was recorded 0.1087 to 6.241 at P > 0.05 between Northern Khobar vs Southern Khobar.

The highest 95% CI of diff was recorded 3.434 to 9.566 at P < 0.001 between Ad-dammam vs Southern Khobar.

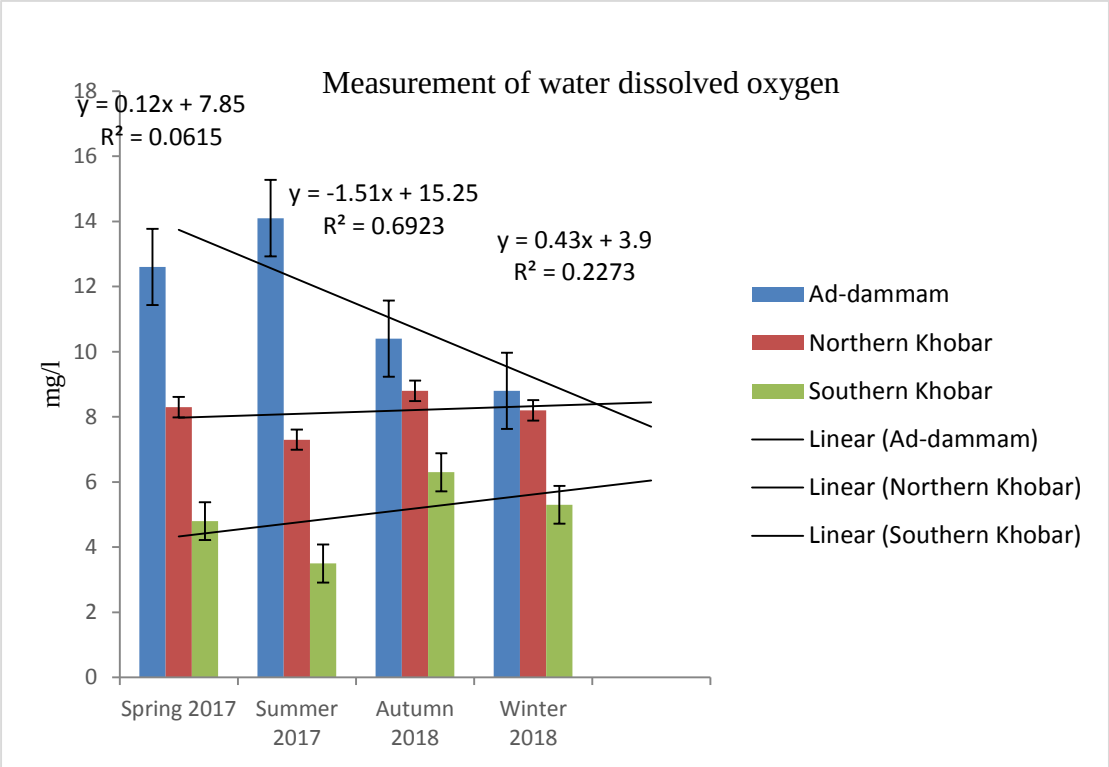

Figure 1g

Figure 1: measurement of water quality. a. temperature ^{o}C. b. water salinity g/l . c. water conductivity (S/m). d. water turbidity NTU . e. water total hardess g/l . f. water PH g/l . g. water dissolved oxygen g/l . h. water chloride (µg/g H_2O , $\mu g g^{-1}$).

Table Analyzed 1g				
dissolved oxygen				
One-way analysis of variance				
P value	0,0008			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	3			
F	17,52			
R squared	0,7957			
ANOVA Table	SS	df	MS	
Treatment (between columns)	84,51	2	42,26	
Residual (within columns)	21,71	9	2,412	
Total	106,2	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Ad-dammam vs Northern Khobar	3,325	4,282	P < 0.05	0.2587 to 6.391
Ad-dammam vs Southern Khobar	6,5	8,371	P < 0.001	3.434 to 9.566
Northern Khobar vs Southern Khobar	3,175	4,089	P < 0.05	0.1087 to 6.241

3.8. Chlorine

The chlorine values are given in (Figure 1h & Table 1h) for the three pan estuaries during the four seasons. Chlorine value of ad-Dammam water ranged from 1700 ± 3.5 to 2000 ± 4. 9 g/l, N Khobar 1660 ± 5.40 to 1989 ± 5.60 and S Khobar 2440 ± 3.61 to 2800 ± 3.7. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's Multiple Comparison Test showed Mean Diff. -10,38 at P < 0.05 between ad-Dammam crab vs S Khobar water, -9,275 at P > 0. 001 between ad- Dammam water vs S Khobar water and 1,1at P < 0.05 between ad- Dammam water vs ad-Dammam crab. The lowest 95% CI of diff was recorded -14.03 to -6.722 at P > 0.001 between ad-Dammam crab vs S Khobar water. The highest 95% CI of diff was recorded -2.553 to 4.753 at P < 0.05 between ad- Dammam water vs ad-Dammam crab.

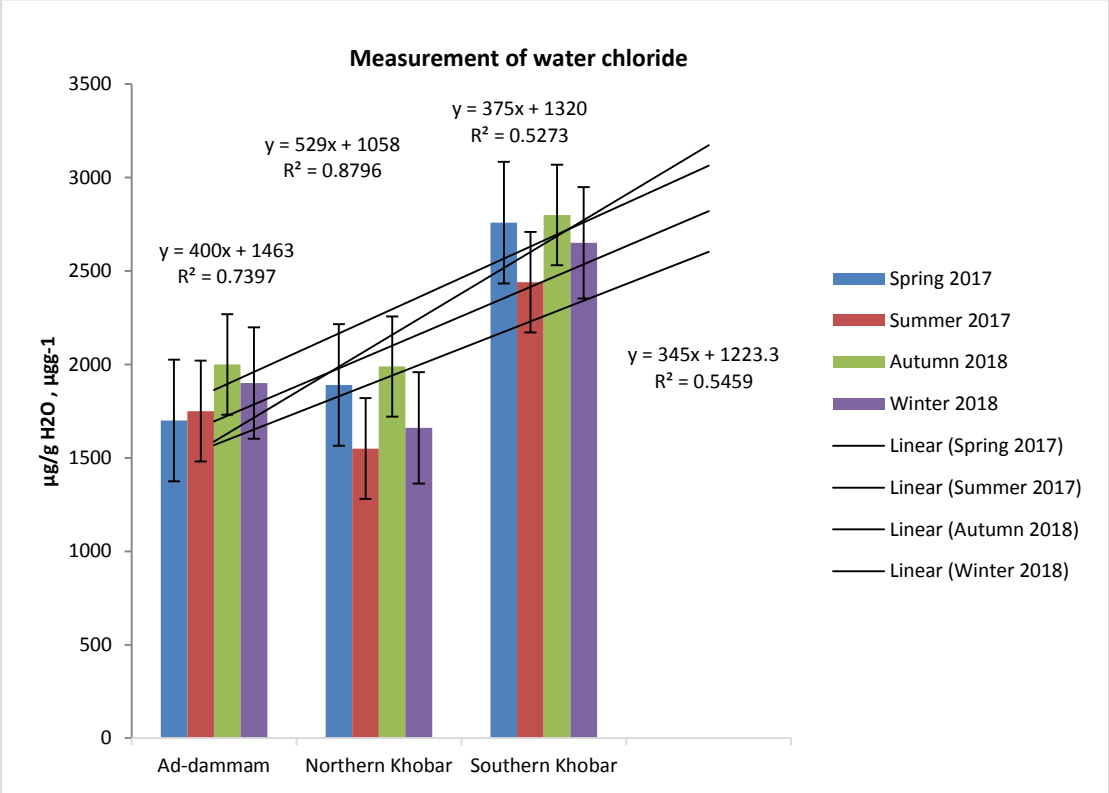

Figure 1h

Table Analyzed 1h				
Chlorine				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	20,9			
R squared	0,853			
ANOVA Table	SS	df	MS	
Treatment (between columns)	276	5	55,21	
Residual (within columns)	47,55	18	2,642	
Total	323,6	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
ad- Dammam water vs ad-Dammam crab	1,1	1,354	P > 0.05	-2.553 to 4.753
ad- Dammam water vs N Kohbar water	-2,575	3,169	P > 0.05	-6.228 to 1.078
ad- Dammam water vs N Khobar crab	-2,95	3,63	P > 0.05	-6.603 to 0.7030
ad- Dammam water vs S Khobar water	-9,275	11,41	P < 0.001	-12.93 to -5.622
ad- Dammam water vs S Khobar crab	-4,925	6,06	P < 0.01	-8.578 to -1.272
ad-Dammam crab vs N Kohbar water	-3,675	4,522	P < 0.05	-7.328 to -0.02200
ad-Dammam crab vs N Khobar crab	-4,05	4,984	P < 0.05	-7.703 to -0.3970
ad-Dammam crab vs S Khobar water	-10,38	12,77	P < 0.001	-14.03 to -6.722
ad-Dammam crab vs S Khobar crab	-6,025	7,414	P < 0.001	-9.678 to -2.372
N Kohbar water vs N Khobar crab	-0,375	0,4614	P > 0.05	-4.028 to 3.278
N Kohbar water vs S Khobar water	-6,7	8,244	P < 0.001	-10.35 to -3.047
N Kohbar water vs S Khobar crab	-2,35	2,892	P > 0.05	-6.003 to 1.303
N Khobar crab vs S Khobar water	-6,325	7,783	P < 0.001	-9.978 to -2.672
N Khobar crab vs S Khobar crab	-1,975	2,43	P > 0.05	-5.628 to 1.678
S Khobar water vs S Khobar crab	4,35	5,353	P < 0.05	0.6970 to 8.003

3.9. Measurement of copper in estuarine water and crab gills

Copper concentrations in mixed gills of crab ranged from 1.01 ± 0.32 to 12.6 ± 1.0 mg/Kg dry weight in samples of Ad-Dammam, 1.06 ± 0.044 to 9.2 ± 0.013 mg/Kg dry weight in samples of Northern Khobar whereas 7.04 ± 0.27 to 26.2 ± 0.063 mg/Kg dry weight in samples of southern Khobar. Figure (2a) & (Table 2a) clarify that copper contamination in crab gills is Ad-Dammam ≈ Northern Khobar < Southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Copper contamination in the three estuaries was Ad-Dammam ≈

Northern Khobar << Southern Khobar. Tukey's multiple comparison test showed Mean Diff. -19,21 at P > 0.01 between crab Dammam vs water S Khobar , 5,2 at P > 0.05 between water Dammam vs crab N Khobar, -14,98 at P > 0.05 between water Dammam vs water S Khobar. However, Copper contamination in sea water of S Khobar beach is highly remarkable.

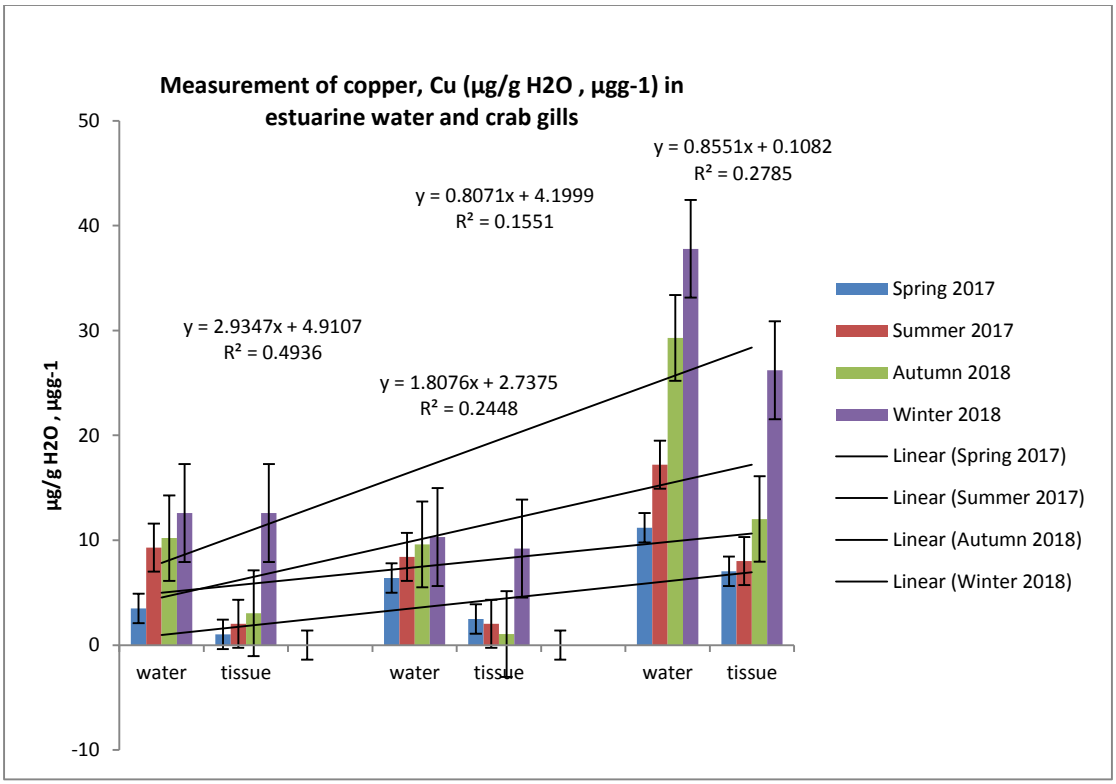

Figure 2a

Table Analyzed 2a			
Measurement of copper			
One-way analysis of variance			
P value	0,0067		
P value summary	**		
Are means Signif. Diff.? (P < 0.05)	Yes		
Number of groups	6		
F	4,655		
R squared	0,5639		
ANOVA Table	SS	df	MS
Treatment (between columns)	1092	5	218,4
Residual (within columns)	844,4	18	46,91
Total	1936	23	

Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	4,23	1,235	P > 0.05	-11.16 to 19.62
water Dammam vs water N Khobar	0,225	0,0657	P > 0.05	-15.17 to 15.62
water Dammam vs crab N Khobar	5,2	1,518	P > 0.05	-10.19 to 20.59
water Dammam vs water S Khobar	-14,98	4,373	P > 0.05	-30.37 to 0.4185
water Dammam vs crab S Khobar	-4,423	1,291	P > 0.05	-19.82 to 10.97
crab Dammam vs water N Khobar	-4,005	1,169	P > 0.05	-19.40 to 11.39
crab Dammam vs crab N Khobar	0,97	0,2832	P > 0.05	-14.42 to 16.36
crab Dammam vs water S Khobar	-19,21	5,608	P < 0.01	-34.60 to -3.811
crab Dammam vs crab S Khobar	-8,653	2,527	P > 0.05	-24.05 to 6.741
water N Khobar vs crab N Khobar	4,975	1,453	P > 0.05	-10.42 to 20.37
water N Khobar vs water S Khobar	-15,2	4,438	P > 0.05	-30.59 to 0.1935
water N Khobar vs crab S Khobar	-4,648	1,357	P > 0.05	-20.04 to 10.75
crab N Khobar vs water S Khobar	-20,18	5,891	P < 0.01	-35.57 to -4.781
crab N Khobar vs crab S Khobar	-9,623	2,81	P > 0.05	-25.02 to 5.771
water S Khobar vs crab S Khobar	10,55	3,081	P > 0.05	-4.841 to 25.95

3.10. Measurement of Zinc in estuarine water and crab gills

Zinc concentrations in mixed gills of crab ranged from 32.32 ± 0.77 to 46.2 ± 0.63 mg/Kg dry weight in samples of Ad-Dammam, 38.74 ± 1,2 to 68.02 ± 0.3 mg/Kg dry weight in samples of Northern Khobar whereas 85.12± 1.2 to 271,07 ± 0.52 mg/Kg dry weight in samples of Southern Khobar. Figure (2b) & (Table 2b) clarify that zinc contamination in crab gills Ad-Dammam < Northern Khobar < southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test showed Mean Diff. 106,1 at P > 0.05 between water S Khobar vs crab S Khobar, 64,28 at P > 0.05 between water Dammam vs crab Dammam, -84,91 at P > 0.05 between crab Dammam vs water N Khobar. However, Zinc contamination in estuarine water and crab gills of S Khobar is highly remarkable.

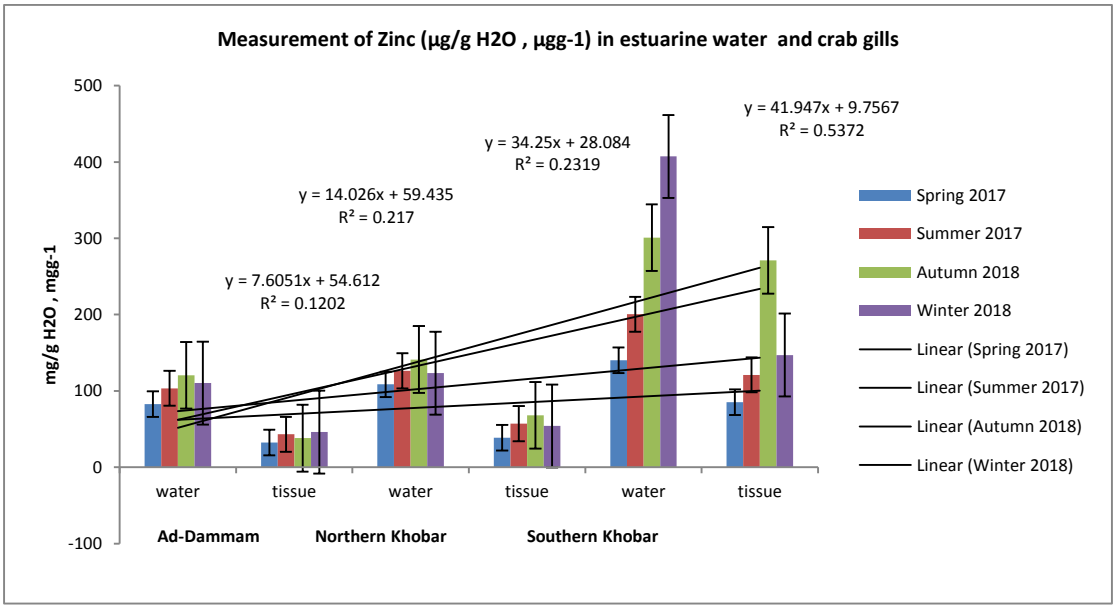

Figure 2b

Table Analyzed 2b				
Measurement of Zinc				
One-way analysis of variance				
P value	0,0006			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	7,447			
R squared	0,6741			
ANOVA Table	SS	df	MS	
Treatment (between columns)	129700	5	25930	
Residual (within columns)	62680	18	3482	
Total	192300	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	64,28	2,179	P > 0.05	-68.35 to 196.9
water Dammam vs water N Khobar	-20,63	0,699	P > 0.05	-153.3 to 112.0
water Dammam vs crab N Khobar	49,7	1,684	P > 0.05	-82.93 to 182.3
water Dammam vs water S Khobar	-158	5,354	P < 0.05	-290.6 to -25.35
water Dammam vs crab S Khobar	-51,91	1,759	P > 0.05	-184.5 to 80.72
crab Dammam vs water N Khobar	-84,91	2,878	P > 0.05	-217.5 to 47.72
crab Dammam vs crab N Khobar	-14,58	0,4941	P > 0.05	-147.2 to 118.0

crab Dammam vs water S Khobar	-222,3	7,533	P < 0.001	-354.9 to -89.63
crab Dammam vs crab S Khobar	-116,2	3,938	P > 0.05	-248.8 to 16.44
water N Khobar vs crab N Khobar	70,33	2,383	P > 0.05	-62.30 to 203.0
water N Khobar vs water S Khobar	-137,4	4,655	P < 0.05	-270.0 to -4.721
water N Khobar vs crab S Khobar	-31,29	1,06	P > 0.05	-163.9 to 101.3
crab N Khobar vs water S Khobar	-207,7	7,038	P < 0.01	-340.3 to -75.05
crab N Khobar vs crab S Khobar	-101,6	3,444	P > 0.05	-234.2 to 31.02
water S Khobar vs crab S Khobar	106,1	3,595	P > 0.05	-26.57 to 238.7

3.11. Measurement of Cadmium in estuarine water and crab gills

Cadmium concentration in mixed crab gills ranged from 1.01 ± 0.12 to 1.01 ± 0.50 mg/Kg dry weight in samples of Ad-Dammam, 1.06 ± 0.07 to 2.01 ± 0.6 mg/Kg dry weight in samples of Northern Khobar whereas 7.07± 0.01 to 5.07 ± 0.2 mg/Kg dry weight in samples of Southern Khobar. Figure (2c) & Table (2c) clarify that cadmium contamination in crab gills is Ad-Dammam > Northern Khobar << southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test showed Mean Diff. 4,938 at P > 0.001 between water S Khobar vs crab S Khobar, 0 at P > 0.05 between water Dammam vs water N Khobar , -8,508 at P > 0.001 between crab N Khobar vs water S Khobar. However, Cadmium contamination in estuarine water and crab gills of Southern Khobar is highly remarkable.

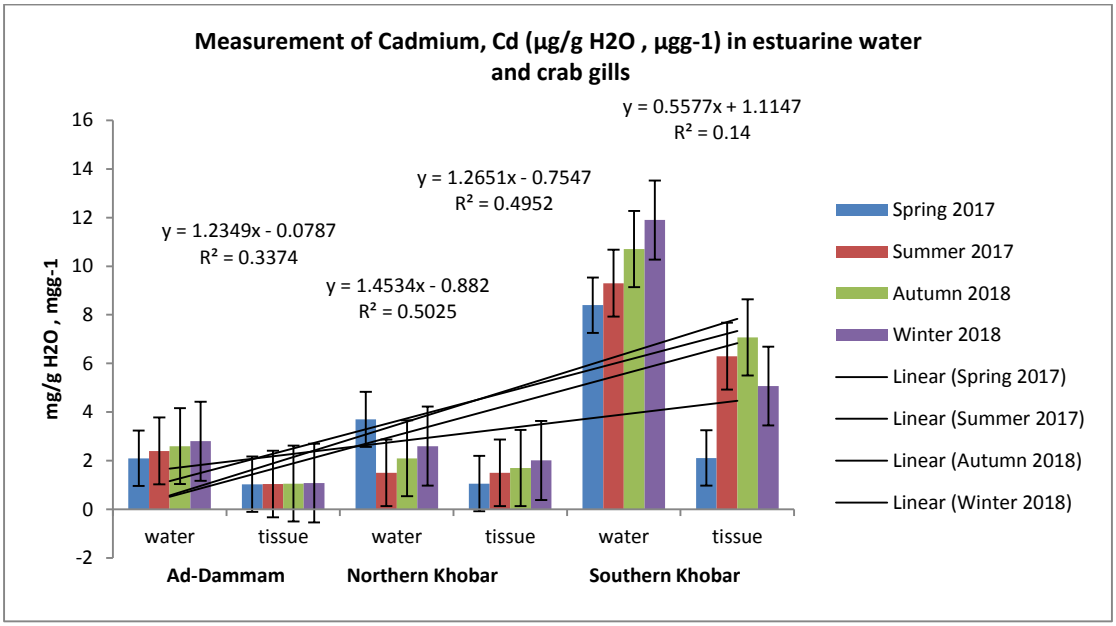

Figure 2c

Table Analyzed 2c				
Measurement of Cadmium				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	33,3			
R squared	0,9025			
ANOVA Table	SS	df	MS	
Treatment (between columns)	228,8	5	45,77	
Residual (within columns)	24,74	18	1,374	
Total	253,6	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	1,423	2,427	P > 0.05	-1.212 to 4.057
water Dammam vs water N Khobar	0	0	P > 0.05	-2.635 to 2.635
water Dammam vs crab N Khobar	0,9075	1,548	P > 0.05	-1.727 to 3.542
water Dammam vs water S Khobar	-7,6	12,97	P < 0.001	-10.23 to -4.965
water Dammam vs crab S Khobar	-2,663	4,542	P < 0.05	-5.297 to -0.02783
crab Dammam vs water N Khobar	-1,423	2,427	P > 0.05	-4.057 to 1.212
crab Dammam vs crab N Khobar	-0,515	0,8786	P > 0.05	-3.150 to 2.120
crab Dammam vs water S Khobar	-9,023	15,39	P < 0.001	-11.66 to -6.388
crab Dammam vs crab S Khobar	-4,085	6,969	P < 0.01	-6.720 to -1.450
water N Khobar vs crab N Khobar	0,9075	1,548	P > 0.05	-1.727 to 3.542
water N Khobar vs water S Khobar	-7,6	12,97	P < 0.001	-10.23 to -4.965
water N Khobar vs crab S Khobar	-2,663	4,542	P < 0.05	-5.297 to -0.02783
crab N Khobar vs water S Khobar	-8,508	14,51	P < 0.001	-11.14 to -5.873
crab N Khobar vs crab S Khobar	-3,57	6,091	P < 0.01	-6.205 to -0.9353
water S Khobar vs crab S Khobar	4,938	8,424	P < 0.001	2.303 to 7.572

3.12. Measurement of lead in estuarine water and crab gills

Lead concentration in mixed crab gills ranged from 2.02 ± 0.2 to 3.03 ± 0.5 mg/Kg dry weight in samples of Ad-Dammam, 1.02 ± 0.,1 to 7.1± 0.9 mg/Kg dry weight in samples of Northern Khobar whereas 3.03 ± 0.8 to 8.8 ± 0.13 mg/Kg dry weight in samples of Southern Khobar. Figure (2d) & (Table (2d) clarify that lead contamination in crab gills is Ad-Dammam > Northern Khobar < southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -6,975 at P > 0.01 between

Anfoushy water vs Abu Qir water , -0,5333 at P > 0.05 between water Dammam vs water S Khobar, -9,37 at P > 0.001 between crab Dammam vs water S Khobar. However, Lead contamination in estuarine water and crab gills of Southern Khobar is highly remarkable.

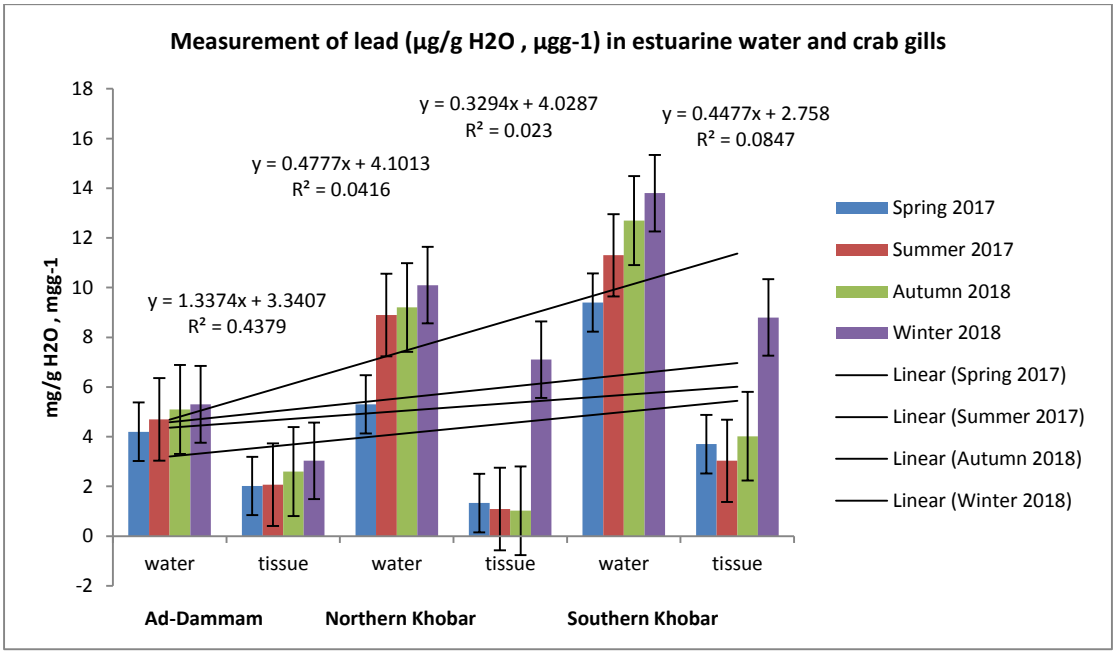

Figure 2d

Table Analyzed 2d			
Measurement of Lead			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means Signif. Diff.? (P < 0.05)	Yes		
Number of groups	6		
F	12,95		
R squared	0,7825		
ANOVA Table	SS	df	MS
Treatment (between columns)	263,1	5	52,63
Residual (within columns)	73,16	18	4,064
Total	336,3	23	

Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	2,395	2,376	P > 0.05	-2.136 to 6.926
water Dammam vs water N Khobar	-3,55	3,522	P > 0.05	-8.081 to 0.9810
water Dammam vs crab N Khobar	2,19	2,173	P > 0.05	-2.341 to 6.721
water Dammam vs water S Khobar	-6,975	6,92	P < 0.01	-11.51 to -2.444
water Dammam vs crab S Khobar	-0,0625	0,062	P > 0.05	-4.593 to 4.468
crab Dammam vs water N Khobar	-5,945	5,898	P < 0.01	-10.48 to -1.414
crab Dammam vs crab N Khobar	-0,205	0,2034	P > 0.05	-4.736 to 4.326
crab Dammam vs water S Khobar	-9,37	9,296	P < 0.001	-13.90 to -4.839
crab Dammam vs crab S Khobar	-2,458	2,438	P > 0.05	-6.988 to 2.073
water N Khobar vs crab N Khobar	5,74	5,694	P < 0.01	1.209 to 10.27
water N Khobar vs water S Khobar	-3,425	3,398	P > 0.05	-7.956 to 1.106
water N Khobar vs crab S Khobar	3,488	3,46	P > 0.05	-1.043 to 8.018
crab N Khobar vs water S Khobar	-9,165	9,092	P < 0.001	-13.70 to -4.634
crab N Khobar vs crab S Khobar	-2,253	2,235	P > 0.05	-6.783 to 2.278
water S Khobar vs crab S Khobar	6,913	6,858	P < 0.01	2.382 to 11.44

3.13. Measurement of Chromium in estuarine water and crab gills

Chromium concentration in mixed crab gills ranged from 0.45 ± 0.43 to 1.1 ± 0.13 mg/Kg dry weight in samples of Ad-Dammam, 0.67 ± 0.23 to 1.7 ± 0.54 mg/Kg dry weight in samples of Northern Khobar whereas 5.09 ± 1.2 to 9.04± 0.98 mg/Kg dry weight in samples of Southern Khobar. Figure (2e) & (Table (2e) clarify that lead contamination in crab gills is Ad-Dammam **>** Northern Khobar << Southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -13,56 at P > 0.001 between crab N Khobar vs water S Khobar 0,475 at P > 0.05 between water Dammam vs water N Khobar, 7,98 at P > 0.001 between water S Khobar vs crab S Khobar. However, chromium contamination in estuarine water and crab gills of Southern Khobar is highly remarkable.

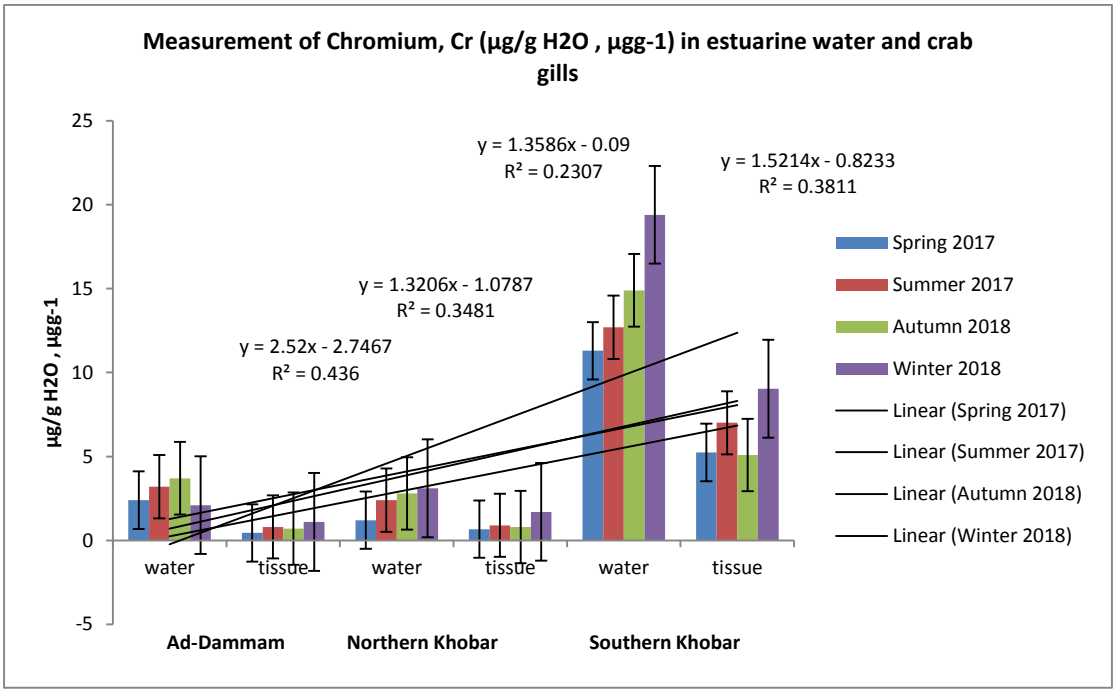

Figure 2e

Table Analyzed 2e				
Measurement of Chromium				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	38,17			
R squared	0,9138			
ANOVA Table	SS	df	MS	
Treatment (between columns)	556	5	111,2	
Residual (within columns)	52,44	18	2,913	
Total	608,4	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	2,088	2,446	P > 0.05	-1.749 to 5.924
water Dammam vs water N Khobar	0,475	0,5566	P > 0.05	-3.361 to 4.311
water Dammam vs crab N Khobar	1,833	2,147	P > 0.05	-2.004 to 5.669
water Dammam vs water S Khobar	-11,73	13,74	P < 0.001	-15.56 to -7.889
water Dammam vs crab S Khobar	-3,745	4,388	P > 0.05	-7.581 to 0.09117

crab Dammam vs water N Khobar	-1,613	1,889	P > 0.05	-5.449 to 2.224
crab Dammam vs crab N Khobar	-0,255	0,2988	P > 0.05	-4.091 to 3.581
crab Dammam vs water S Khobar	-13,81	16,18	P < 0.001	-17.65 to -9.976
crab Dammam vs crab S Khobar	-5,833	6,834	P < 0.01	-9.669 to -1.996
water N Khobar vs crab N Khobar	1,358	1,591	P > 0.05	-2.479 to 5.194
water N Khobar vs water S Khobar	-12,2	14,3	P < 0.001	-16.04 to -8.364
water N Khobar vs crab S Khobar	-4,22	4,945	P < 0.05	-8.056 to -0.3838
crab N Khobar vs water S Khobar	-13,56	15,89	P < 0.001	-17.39 to -9.721
crab N Khobar vs crab S Khobar	-5,578	6,535	P < 0.01	-9.414 to -1.741
water S Khobar vs crab S Khobar	7,98	9,35	P < 0.001	4.144 to 11.82

3.14. Measurement of Aluminum in estuarine water and crab gills Aluminum concentration in mixed crab gills ranged from 1.02 ± 0.71 to 3.9 ± 0.34 mg/Kg dry weight in samples of Ad-Dammam, 1.05 ± 0.45 to 3.1 ± 0.35 mg/Kg dry weight in samples of Northern Khobar whereas 12.6 ± 3.2 to 34.05 ± 4.5 mg/Kg dry weight in samples of Southern Khobar. Figure (2f) & (Table (2f) clarify that lead contamination in crab gills is Ad-Dammam ≈ Northern Khobar << Southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -19,83 at P > 0.05 between crab N Khobar vs water S Khobar, -2,2 at P > 0.05 between water Dammam vs water N Khobar, 8,22 at P > 0.05 between water N Khobar vs crab N Khobar. However, Aluminum contamination in estuarine water and crab gills of Southern Khobar is highly remarkable.

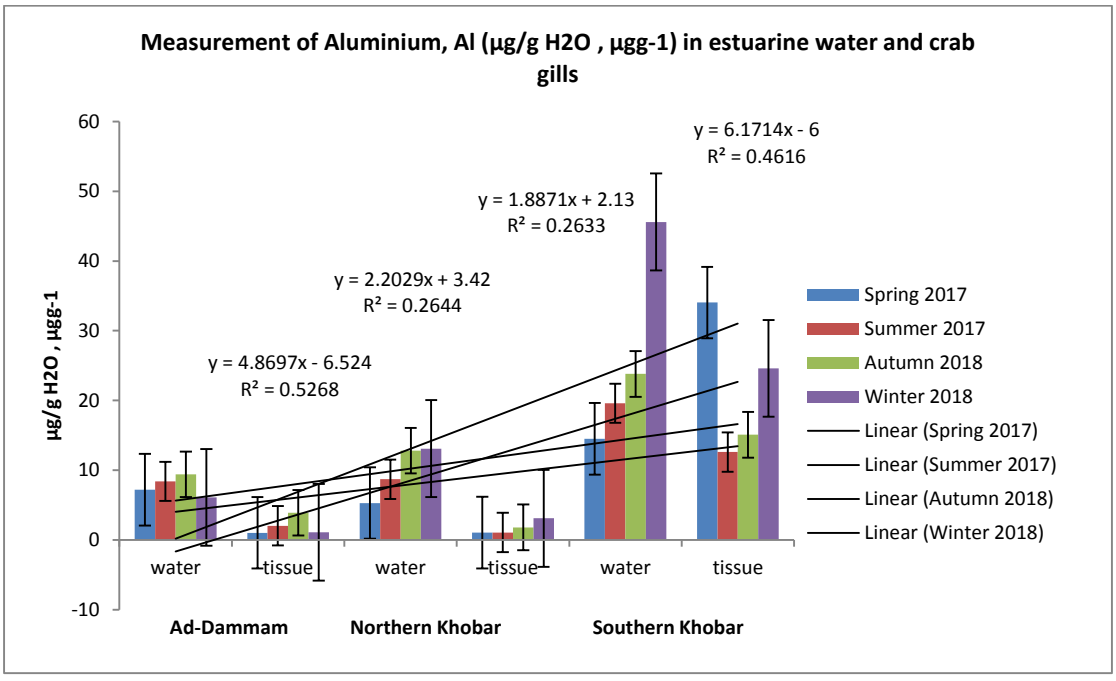

Figure 2f

Table Analyzed 2f				
Measurement of Aluminum				
One-way analysis of variance				
P value	0,0004			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	8,104			
R squared	0,6924			
ANOVA Table	SS	df	MS	
Treatment (between columns)	2038	5	407,5	
Residual (within columns)	905,2	18	50,29	
Total	2943	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	5,76	1,624	P > 0.05	-10.18 to 21.70
water Dammam vs water N Khobar	-2,2	0,6205	P > 0.05	-18.14 to 13.74
water Dammam vs crab N Khobar	6,02	1,698	P > 0.05	-9.918 to 21.96
water Dammam vs water S Khobar	-18,1	5,105	P < 0.05	-34.04 to -2.162
water Dammam vs crab S Khobar	-13,81	3,894	P > 0.05	-29.75 to 2.131
crab Dammam vs water N Khobar	-7,96	2,245	P > 0.05	-23.90 to 7.978
crab Dammam vs crab N Khobar	0,26	0,07333	P > 0.05	-15.68 to 16.20
crab Dammam vs water S Khobar	-23,86	6,729	P < 0.01	-39.80 to -7.922
crab Dammam vs crab S Khobar	-19,57	5,519	P < 0.05	-35.51 to -3.629
water N Khobar vs crab N Khobar	8,22	2,318	P > 0.05	-7.718 to 24.16
water N Khobar vs water S Khobar	-15,9	4,484	P > 0.05	-31.84 to 0.03818
water N Khobar vs crab S Khobar	-11,61	3,274	P > 0.05	-27.55 to 4.331
crab N Khobar vs water S Khobar	-24,12	6,802	P < 0.01	-40.06 to -8.182
crab N Khobar vs crab S Khobar	-19,83	5,592	P < 0.05	-35.77 to -3.889
water S Khobar vs crab S Khobar	4,293	1,211	P > 0.05	-11.65 to 20.23

3.15. Measurement of iron in estuarine water and crab gills

Aluminum concentration in mixed crab gills ranged from 2.1 ± 0.40 to 2.46 ± 0.38 mg/Kg dry weight in samples of Ad-Dammam, 3.1 ± 0.17 to 3.6 ± 0.79 mg/Kg dry weight in samples of Northern Khobar whereas 3.2 ± 1.3 to 4.8 ± 2.1 mg/Kg dry weight in samples of Southern Khobar. Figure (2g) & (Table (2g) clarify that lead contamination in crab gills is Ad-Dammam < Northern Khobar << Southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -1,5 at P > 0.001 between S Khobar water vs S Khobar crab, -2,393 at P > 0.001 between ad-Dammam water

vs "S Khobar crab, -0,455 at P > 0.05 between ad-Dammam water vs N Kohbar water. However, iron contamination in estuarine water and crab gills of Southern Khobar is highly remarkable.

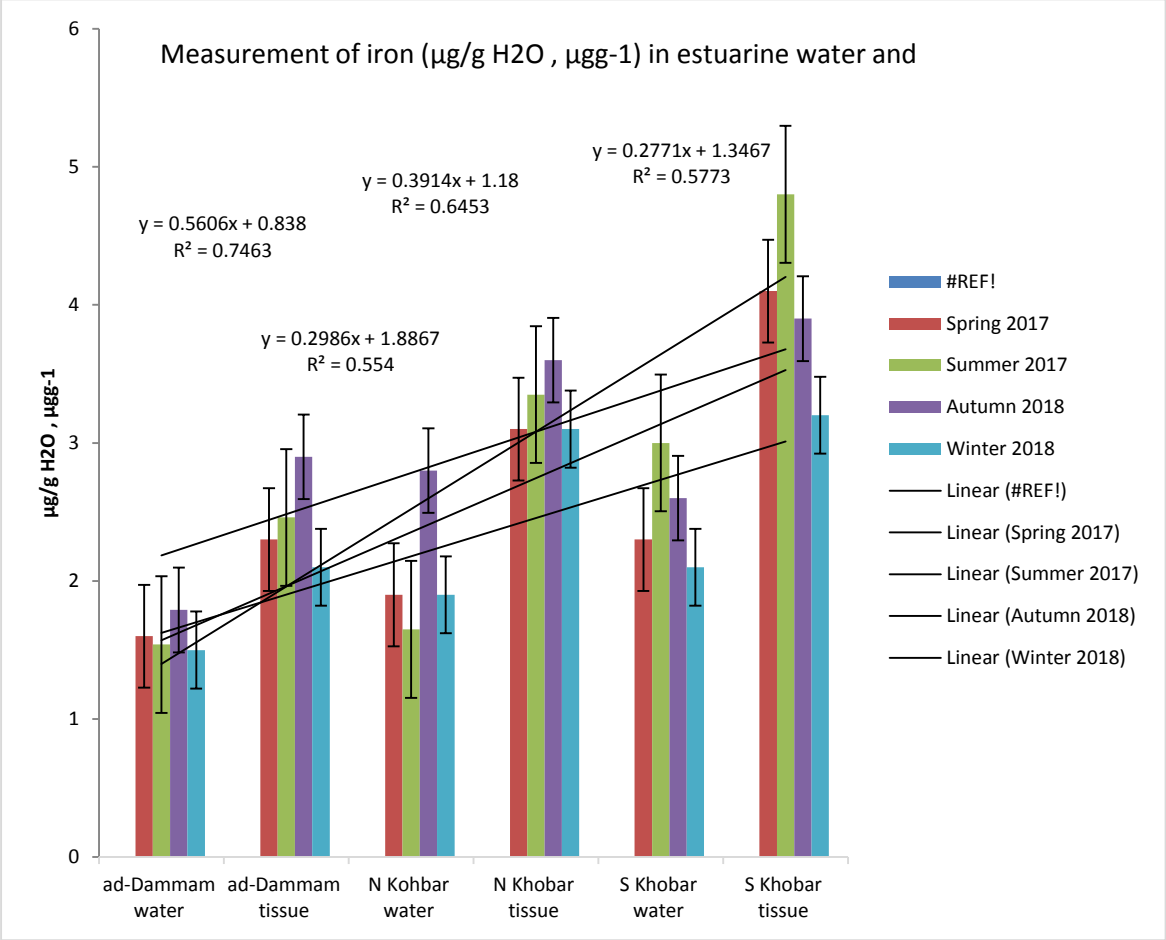

Figure 2g

Table Analyzed 2g	
Iron	
One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means Signif. Diff.? (P < 0.05)	Yes
Number of groups	6
F	17,34

R squared	0,8281			
ANOVA Table	SS	df	MS	
Treatment (between columns)	14,91	5	2,982	
Residual (within columns)	3,095	18	0,172	
Total	18,01	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
ad-Dammam water vs ad-Dammam crab	-0,8325	4,015	P > 0.05	-1.765 to 0.09952
ad-Dammam water vs N Kohbar water	-0,455	2,194	P > 0.05	-1.387 to 0.4770
ad-Dammam water vs N Khobar crab	-1,68	8,102	P < 0.001	-2.612 to -0.7480
ad-Dammam water vs S Khobar water	-0,8925	4,304	P > 0.05	-1.825 to 0.03952
ad-Dammam water vs "S Khobar crab"	-2,393	11,54	P < 0.001	-3.325 to -1.460
ad-Dammam crab vs N Kohbar water	0,3775	1,821	P > 0.05	-0.5545 to 1.310
ad-Dammam crab vs N Khobar crab	-0,8475	4,087	P > 0.05	-1.780 to 0.08452
ad-Dammam crab vs S Khobar water	-0,06	0,2894	P > 0.05	-0.9920 to 0.8720
ad-Dammam crab vs "S Khobar crab"	-1,56	7,524	P < 0.001	-2.492 to -0.6280
N Kohbar water vs N Khobar crab	-1,225	5,908	P < 0.01	-2.157 to -0.2930
N Kohbar water vs S Khobar water	-0,4375	2,11	P > 0.05	-1.370 to 0.4945
N Kohbar water vs "S Khobar crab"	-1,938	9,344	P < 0.001	-2.870 to -1.005
N Khobar crab vs S Khobar water	0,7875	3,798	P > 0.05	-0.1445 to 1.720
N Khobar crab vs "S Khobar crab"	-0,7125	3,436	P > 0.05	-1.645 to 0.2195
S Khobar water vs "S Khobar crab"	-1,5	7,234	P < 0.001	-2.432 to -0.5680

3.16. Measurement of Manganese in estuarine water and crab gills

Manganese concentration in mixed crab gills ranged from 2.2 ± 0.90 to 2.6 ± 0.76 mg/Kg dry weight in samples of Ad-Dammam, 3.6 ± 0.87 to 4.2 ± 0.58 mg/Kg dry weight in samples of Northern Khobar whereas 3.9 ± 0.83 to 5.5 ± 1.3 mg/Kg dry weight in samples of Southern Khobar. Figure (2h) & (Table (2h) clarify that lead contamination in crab gills is Ad-Dammam < Northern Khobar < Southern Khobar. ANOVA test showed Signif. Difference at $P < 0.05$ for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -4,375 at $P > 0.001$ between ad-Dammam crab vs S Khobar water, -0,75 at $P > 0.001$ between ad- Dammam water vs ad-Dammam crab , 2,225 at $P > 0.001$ between S Khobar water vs S Khobar crab. However, manganese contamination in estuarine water and crab gills of Southern

Khobar is highly remarkable.

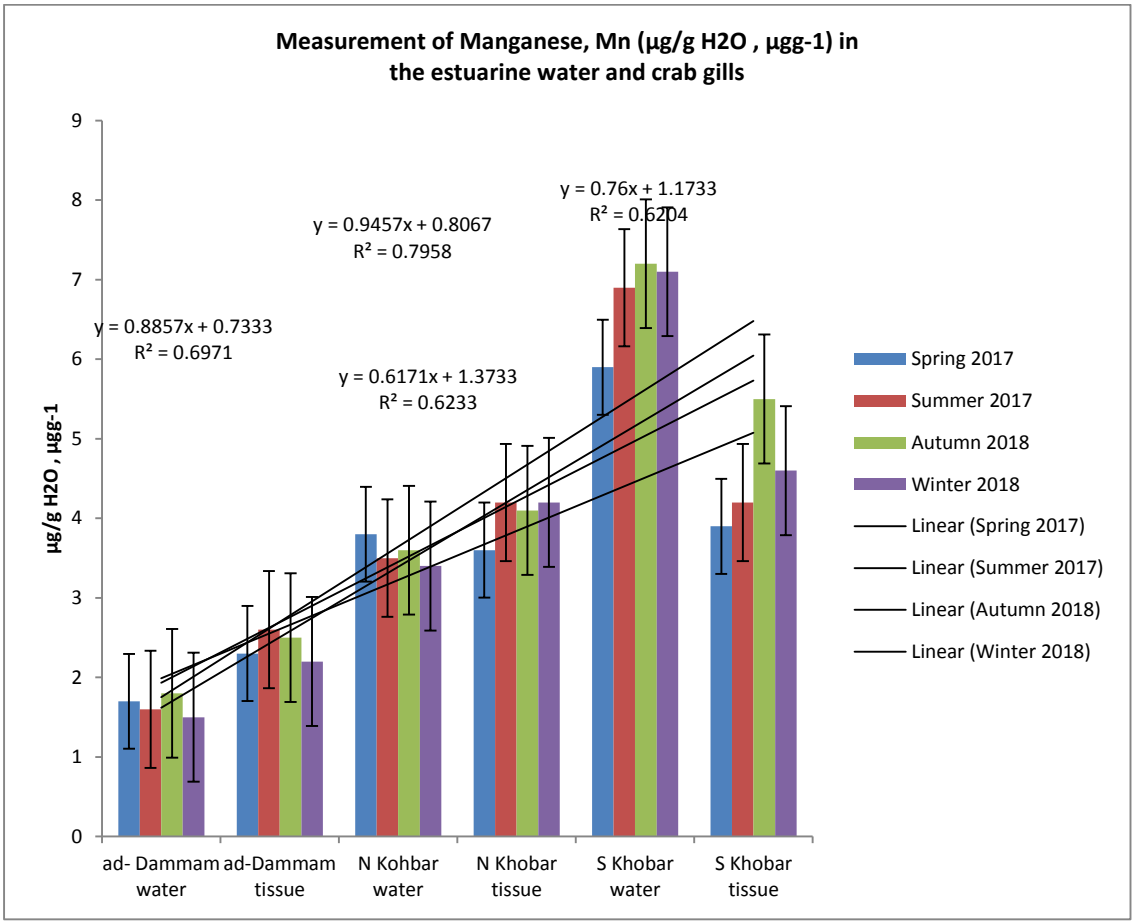

Figure 2h

Table Analyzed 2h			
Manganese			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means Signif. Diff.? (P < 0.05)	Yes		
Number of groups	6		
F	77,18		
R squared	0,9554		
ANOVA Table	SS	df	MS
Treatment (between columns)	64,37	5	12,87
Residual (within columns)	3,002	18	0,1668
Total	67,37	23	

Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
ad- Dammam water vs ad-Dammam crab	-0,75	3,673	P > 0.05	-1.668 to 0.1679
ad- Dammam water vs N Kohbar water	-1,925	9,427	P < 0.001	-2.843 to -1.007
ad- Dammam water vs N Khobar crab	-2,375	11,63	P < 0.001	-3.293 to -1.457
ad- Dammam water vs S Khobar water	-5,125	25,1	P < 0.001	-6.043 to -4.207
ad- Dammam water vs S Khobar crab	-2,9	14,2	P < 0.001	-3.818 to -1.982
ad-Dammam crab vs N Kohbar water	-1,175	5,754	P < 0.01	-2.093 to -0.2571
ad-Dammam crab vs N Khobar crab	-1,625	7,958	P < 0.001	-2.543 to -0.7071
ad-Dammam crab vs S Khobar water	-4,375	21,42	P < 0.001	-5.293 to -3.457
ad-Dammam crab vs S Khobar crab	-2,15	10,53	P < 0.001	-3.068 to -1.232
N Kohbar water vs N Khobar crab	-0,45	2,204	P > 0.05	-1.368 to 0.4679
N Kohbar water vs S Khobar water	-3,2	15,67	P < 0.001	-4.118 to -2.282
N Kohbar water vs S Khobar crab	-0,975	4,775	P < 0.05	-1.893 to -0.05708
N Khobar crab vs S Khobar water	-2,75	13,47	P < 0.001	-3.668 to -1.832
N Khobar crab vs S Khobar crab	-0,525	2,571	P > 0.05	-1.443 to 0.3929
S Khobar water vs S Khobar crab	2,225	10,9	P < 0.001	1.307 to 3.143

3.17. Measurement of Nickel in estuarine water and crab gills

Nickel concentration in mixed crab gills ranged from 1.04 ± 0.60 to 1.1 ± 0.27 mg/Kg dry weight in samples of Ad-Dammam, 1.2 ± 0.21 to 2.7 ± 0.85 mg/Kg dry weight in samples of Northern Khobar whereas 4.05± 1.10 to 13.3 ± 2.30 mg/Kg dry weight in samples of Southern Khobar. Figure (2i) & (Table (2i) clarify that lead contamination in crab gills is Ad-Dammam ≈ Northern Khobar >> Southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -13,32 at P > 0.001 between crab N Khobar vs water S Khobar, -0,85 at P > 0.05 between water Dammam vs water N Khobar, 1,143 at P > 0.05 between water Dammam vs crab N Khobar. However, Nickel contamination in estuarine water and crab gills of Southern Khobar is highly remarkable.

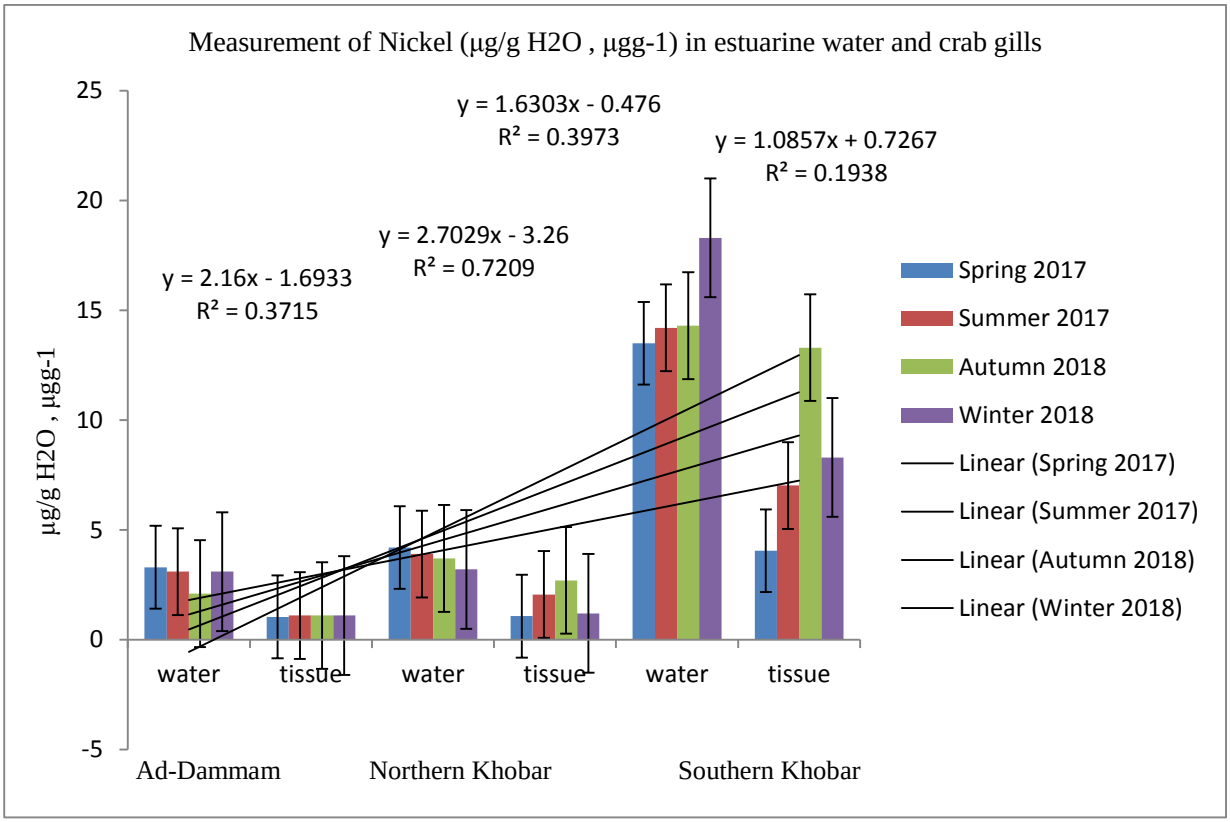

Figure 2i

Table Analyzed 2i				
Measurement of Nickel				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	32,98			
R squared	0,9016			
ANOVA Table	SS	df	MS	
Treatment (between columns)	568,4	5	113,7	
Residual (within columns)	62,05	18	3,447	
Total	630,5	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	1,815	1,955	P > 0.05	-2.358 to 5.988
water Dammam vs water N Khobar	-0,85	0,9156	P > 0.05	-5.023 to 3.323
water Dammam vs crab N Khobar	1,143	1,231	P > 0.05	-3.031 to 5.316
water Dammam vs water S Khobar	-12,18	13,11	P < 0.001	-16.35 to -8.002
water Dammam vs crab S Khobar	-5,267	5,674	P < 0.01	-9.441 to -1.094
crab Dammam vs water N Khobar	-2,665	2,871	P > 0.05	-6.838 to 1.508
crab Dammam vs crab N Khobar	-0,6725	0,7244	P > 0.05	-4.846 to 3.501
crab Dammam vs water S Khobar	-13,99	15,07	P < 0.001	-18.16 to -9.817

crab Dammam vs crab S Khobar	-7,083	7,629	P < 0.001	-11.26 to -2.909
water N Khobar vs crab N Khobar	1,993	2,146	P > 0.05	-2.181 to 6.166
water N Khobar vs water S Khobar	-11,33	12,2	P < 0.001	-15.50 to -7.152
water N Khobar vs crab S Khobar	-4,418	4,758	P < 0.05	-8.591 to -0.2445
crab N Khobar vs water S Khobar	-13,32	14,35	P < 0.001	-17.49 to -9.144
crab N Khobar vs crab S Khobar	-6,41	6,905	P < 0.01	-10.58 to -2.237
water S Khobar vs crab S Khobar	6,908	7,44	P < 0.001	2.734 to 11.08

3.18. Measurement of Calcium in estuarine water and crab gills

Calcium concentration in mixed crab gills ranged from 13,4 ± 2.40 to 20,7 ± 3.50 mg/Kg dry weight in samples of Ad-Dammam 14,8 ± 4.10 to 20,1 ± 2.50 mg/Kg dry weight in samples of Northern Khobar whereas 17 ± 3.20 to 18.2 ± 3.80 mg/Kg dry weight in samples of Southern Khobar. Figure (2j) & (Table (2j) clarify that lead contamination in crab gills is Ad-Dammam ≈ Northern Khobar >> Southern Khobar. ANOVA test showed Signif. Difference at P < 0.05 for the three pan estuaries. Tukey's multiple comparison test shows Mean Diff. -8,125 at P > 0.001 between water S Khobar vs crab S Khobar, -1,6 at P > 0.01 between crab Dammam vs crab S Khobar, 2,45 at P > 0.05 between water Dammam vs water S Khobar. However, Calcium contamination in the three estuaries water and crab gills is highly remarkable.

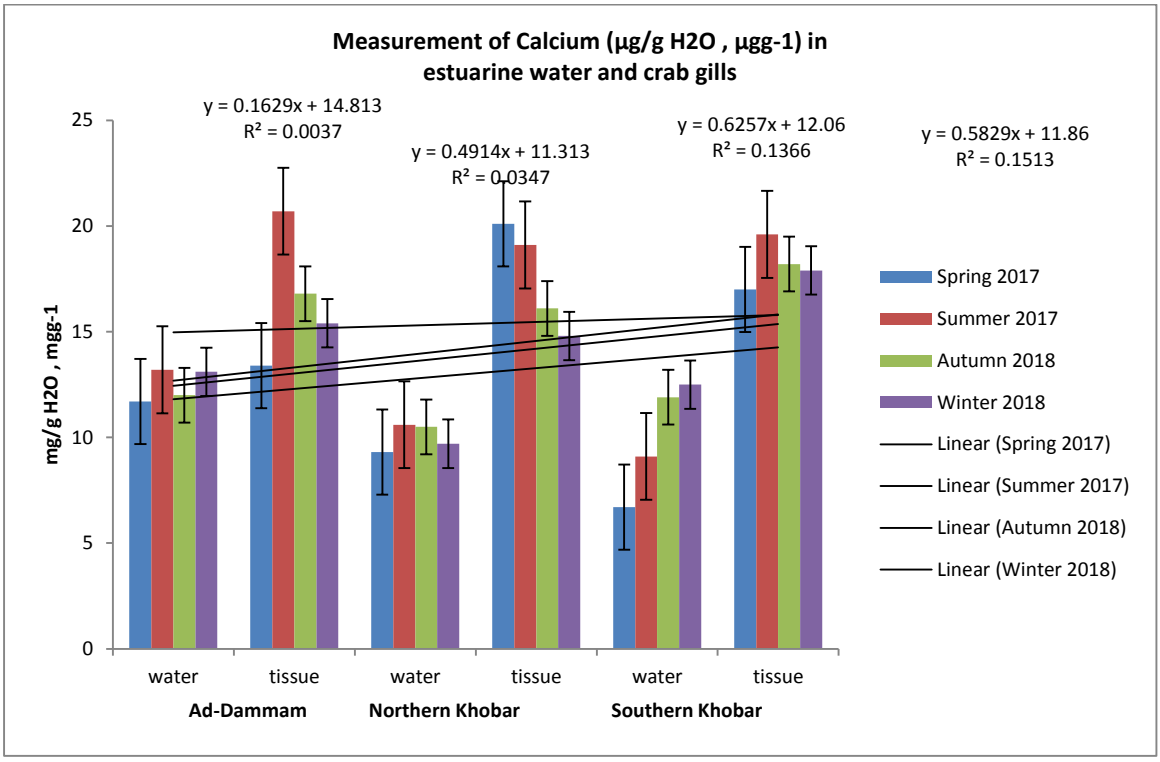

Figure 2j

Figure 2: Measurement of heavy metals in estuarine water (µg/g H_2O) and crab gills (μgg^{-1}) . **a.** Cu . **b.** Zn. **c.** Cd. **d.** Pb. **e.** Cr . **f.** Al. **g.** Fe. **h.** Mn. **i.** Ni. **j.** Ca.

Table Analyzed 2j				
Measurement of Calcium				
One-way analysis of variance				
P value	P<0.0001			
P value summary	***			
Are means Signif. Diff.? (P < 0.05)	Yes			
Number of groups	6			
F	13,43			
R squared	0,7886			
ANOVA Table	SS	df	MS	
Treatment (between columns)	280,1	5	56,02	
Residual (within columns)	75,06	18	4,17	
Total	355,1	23		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
water Dammam vs crab Dammam	-4,075	3,991	P > 0.05	-8.665 to 0.5145
water Dammam vs water N Khobar	2,475	2,424	P > 0.05	-2.115 to 7.065
water Dammam vs crab N Khobar	-5,025	4,922	P < 0.05	-9.615 to -0.4355
water Dammam vs water S Khobar	2,45	2,4	P > 0.05	-2.140 to 7.040
water Dammam vs crab S Khobar	-5,675	5,558	P < 0.05	-10.26 to -1.085
crab Dammam vs water N Khobar	6,55	6,415	P < 0.01	1.960 to 11.14
crab Dammam vs crab N Khobar	-0,95	0,9304	P > 0.05	-5.540 to 3.640
crab Dammam vs water S Khobar	6,525	6,391	P < 0.01	1.935 to 11.11
crab Dammam vs crab S Khobar	-1,6	1,567	P > 0.05	-6.190 to 2.990
water N Khobar vs crab N Khobar	-7,5	7,346	P < 0.001	-12.09 to -2.910
water N Khobar vs water S Khobar	-0,025	0,02448	P > 0.05	-4.615 to 4.565
water N Khobar vs crab S Khobar	-8,15	7,982	P < 0.001	-12.74 to -3.560
crab N Khobar vs water S Khobar	7,475	7,321	P < 0.001	2.885 to 12.06
crab N Khobar vs crab S Khobar	-0,65	0,6366	P > 0.05	-5.240 to 3.940
water S Khobar vs crab S Khobar	-8,125	7,958	P < 0.001	-12.71 to -3.535

4. DISCUSSION

An ideal biomonitoring species must fulfill several criteria: easily identified, relatively abundant, cosmopolitan, hardly enough to survive under high metals contamination, long-lived and available throughout the year, sufficient size to allow analysis and be net accumulators of the metal of interest [50]. Crabs are considered as rich nutritive crustaceans due to the mineral content (phosphorus, calcium, iron and iodine), glycogen, vitamins (A, B1, B2, C and D7) and protein. Aquatic organisms may be exposed to heavy metals dissolved in the ambient water, either from natural

sources or as pollutants released of anthropogenic activities such as mining or industrial processes [22]. They may take up these metals, and have the potential to accumulate them to high concentrations [46, 47]. Environmental research efforts have revealed that many marine invertebrates accumulate metals in their gills from the environment [34, 51]. The metals might be taken up directly from the surrounding aquatic medium or they may be ingested via food particles or contaminated prey items. Therefore, the relative proportion from each route varies with the invertebrate type and the relative availabilities of the metal in the water and diet [32, 50]. Decapod crustaceans absorb heavy metals from their food sources and their permeable body surfaces such as the gills [52]. [53] measured copper, nickel. Lead and zinc by snail, *Lunella coronatus* and pearl oyster, *Pinctada radiata* from the Kuwait coast before and after the Gulf war oil spill. This study found that the levels of Cu and Pb were higher than the [54] acceptable limits for marine organisms. Selected heavy metals were measured in the edible clam *Meretrix meretrix* collected from stations along the coastline of Saudi Arabia [7]. Elevated level of Pb that exceeded the maximum permissible level recommended by the European Union standards. Nonetheless, this study concluded that the clam from the sampling region was within the safe limits for human consumption. [6] determined the levels of selected heavy metals in the cuttlefish *Sepia pharaonis* collected from different fish markets at Al-Khobar City in the Arabian Gulf. This study concluded that the levels of the investigated heavy metals in the cuttlefish were generally low and/or well within the maximum permitted concentrations imposed by different organizations and authorities, and consequently within the safe limits for human consumption. This study agreed with the finding of [6], as *Sepia pharaonis* is not a filter feeder as clams. [55] studied Cu, Zn, Pb, Cd and As accumulations in four species of fish and one species of shrimp from Dammam - Qatif Coast, Arabian Gulf during winter and summer 2012. They found that the highest concentrations of physical and chemical characteristics in water and sediment were as a result of waste water drainage. The average concentration of studied heavy metals in sediment were 0.33, 0.42, 0.25, 0.16 and 0.031ppm which were higher than that recorded in water (0.23, 0.35, 0.0.12, 0.09 and 0.017 ppm), respectively during summer. More or less similar results were recorded in fishes and crustaceans of the Gulf of Khambhat, India [56]. In the present study, the values of salinity g/l, conductivity in Siemens per meter (S/m), water turbidity in NTU, total hardness g/l and water PH in mg/l are within the recommended range CONAMA Resolution No. 357/2005 European Union standards [2], Saudi Arabian Standards [3] ,WHO [4].. However, in contrast to the previous parameters, data obtained from dissolved oxygen (DO) prove that in Southern Khobar estuary reasonably oxygenation occurs but already moving towards worrying levels. Losses of this element is due to consumption processes during the oxidation of organic matter, losses to the atmosphere and the respiration of organisms and oxidation of metallic ions. In the present study, the collected data showed very high concentration of chlorine, copper, zinc, Cadmium, lead, Chromium, Aluminum, iron, Manganese, Nickel, calcium especially in South Khobar estuary water and crab gills which can cause significant adverse effects on the crustacean species if concentrations continue to accumulate and bio-magnify in the food chain. According to European Union standards [2], Saudi

Arabian Standards [3] ,WHO [4]: DO 2.0-3.4 mg/l, PH 6.0–8.4 mg/l , Turbidity 1.0 - 4.7 NTU, Salinity43.64- 54.21 g/l, the saved limits of heavy ranges (in dry weight ppm): copper 31.2 – 73.0 ppm, zinc 4.6 – 180.5 ppm, Cadmium11.7 – 199.1 ppm, lead 0.25 – 0.75 ppm, Chromium 0.0154–0.0184 ppm, Aluminum 0.5-1.9, iron 0.023–0.049 ppm, Manganese 0.0608–0.199 ppm, Nickel 0.3312- 1.8630ppm. The most interesting work concerning heavy metals accumulations in marine animals inhabiting the Arabian Gulf is [6]. In this study, the Arabian Gulf environmental status was assessed based on studies conducted in Bahrain, Kuwait, Oman, Saudi Arabia, Qatar, and United Arab Emirates (UAE) during 1983 and 2011. The present study accept the finding of [13, 25, 28, 38, 57] and disagree with the results of [6]. Levels of selected heavy metals in *Pinctada radiata* collected from two oyster beds in Bahrain were determined by [57]. This study reported elevated levels of Pb and Cd that exceeded the recommended standards of the World Health Organization [4]. Similarly, elevated levels of heavy metals were reported in *P. radiata* collected from areas that were subject to dredging and shipping activities along the Qatari coastline [9]. This study reported high mean concentrations for V exceeded the international standards. [38] also found very high concentrations of Zn in pearl oysters near the oil refinery in Bahrain.

This study concludes that crabs live in Southern Khobar estuary contain percentage of heavy metals ≥ Saudi Arabian Standards whereas that live the Northern Khobar and Ad-dammam estuaries contain percentage of heavy metals ≈ Saudi Arabian Standards. Heavy metal accumulations present in the gills of fishes from Arabian Gulf were reported. Mercury and other metals, such as lead and cadmium have been shown to accumulate in living organisms living in marine ecosystems [58]. Metal accumulation by the clam *Meretrix meretrix* as lead, titanium, zinc, nickel, vanadium and copper were measured in Arabian Gulf [7, 59] The EDXRF technique is well suited for multi-element determinations in environmental samples. Cadmium (Cd) is one of the most toxic heavy metals for humans, the main source of non-occupational exposure to Cd includes contaminated sea foods [37, 60, 61]. The effects of contaminants on aquatic communities are used to indicate changes in environmental quality and conditions [62]. These responses might be behavioral, physiological, histopathological, biochemical or immunological [63] or may affect other aspects of the biology of the organisms of choice. Crabs in particular act as appropriate indicator organisms due to certain factors, including abundance in numbers and biomass, as well as relatively low mobility compared to other marine organisms such as fish [64]. Heavy metal content differs significantly among different organs in a freshwater crab, *Potamonautes perlatus* [65] and also in the marine Norway lobster, *Nephrops norvegicus*. Lobsters from an area with relatively high concentrations of manganese were found to accumulate a significantly higher concentration of the metal in the body, especially in the carapace, while muscle gills showed the lowest concentration [51]. Different metals show variable behaviors in bioaccumulation, for example they may be accumulated at different concentrations in different tissues. In the shore crab *Carcinus maenas* exposed to metals for 32 days, cadmium accumulates primarily in midgut gland and gills, copper in gills and zinc in gill muscles [64]. In the same experimental study, concentrations of cadmium in different tissues seem to reflect the

exposure of the crabs to this metal, whereas concentrations of copper and zinc did not reflect the exposures. When the mangrove crab *Ucides cordatus* was exposed to Mn in sea water, the metal accumulated in different tissues in proportion to the exposure concentration, but to different absolute levels highest in the gills, followed by the hepatopancreas, and least in the gill muscles [45]. Different species accumulate metals at different rates, depending on how they handle the metals (Rainbow, 2001, Mohorjy & Khan, 2006). For marine crustaceans, metal accumulation processes vary not only between genera, but also between closely related species, some being net accumulators, while others are regulators [17]. Net accumulation occurs when the rate of uptake into an organism exceeds the rate of excretion. Metal bioaccumulation can also be affected by the individual size of the organism. Smaller animals accumulate more metals in both fresh water crabs [65] and marine crabs [66]. However the statement does not apply to all taxa. No significant relationship was found between the size of crustaceans, *Acanthephyra eximia*, *Aristeus antennatus* and *Polycheles typhlops* and total body manganese concentrations [20]. [30] found no difference in metal accumulation (of Cd, Pb and Zn, and also Mn) between sexes in three different crab species. However, other studies reported that the accumulation does vary significantly between male and female animals (lobster *Nephrops norvegicus* – [36, 43], crab *Potamonautes warreni* – [67], shrimp *Pleoticus muelleri* – [68]. The World Health Organization [4] estimated that 80% of the world population presently uses herbal medicine. Several articles have reported of adverse effects of these herbal preparations due to the presence of high level of heavy metals such as Cd, lead, chromium, nickel, etc. [69]. The results revealed that the concentrations of some heavy metals, including Cd, were far greater than the permissible limits proposed by the International Regulatory Authorities for herbal drugs. Acute or chronic exposure of Cd causes respiratory distress, lung, breast and endometrial cancers, cardiovascular disorders and endocrine dysfunction [6, 32]. In addition, Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources [19]. It can be accumulated in aquatic animals (crabs, shrimps, oysters, clams and mussels) after entering through different way such as respiratory tract, digestive tract, surface penetration etc. [9, 12, 16, 33, 45, 70]. It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, these animals exposed to Cd might threaten human health.

5. CONCLUSION

Values of salinity g/l, conductivity in Siemens per meter (S/m), water turbidity in NTU, total hardness g/l and water PH in mg/l are within the recommended range CONAMA Resolution No. 357/2005 (European Union standards [2], Saudi Arabian Standards [3] ,WHO [4]. The most dramatic results came with the analysis of the samples of the southern Khobar estuary in the four seasons. The collected data show high concentration of Chlorine, copper, Zinc, Cadmium, lead, Chromium, Aluminum, Iron, Manganese, Nickel and Calcium in ($\mu g/g$ H_2O , $\mu g g^{-1}$) in sea water and crab gills. This contamination can cause significant adverse effects on the crustacean

species if concentrations continue to accumulate and bio-magnify in the food chain. Crabs live in Southern Khobar estuary contain percentage of heavy metals ≥ Saudi Arabian Standards whereas crabs live the Northern Khobar and Ad-dammam estuaries contain percentage of heavy metals ≈ Saudi Arabian Standards. The accumulation of pollutants can have a synergistic effect that can increase the threat level exponentially, but each single metal can impact the organism alone. The Northern Khobar estuary contains moderate amount of heavy metals except iron and manganese whereas Ad-Dammam estuary lies within Saudi Arabian Standards. The presence of these identified heavy metals present in the crab gills indicates serious problems within the aquatic biota of Arabian Gulf. Different marine species react to metal toxicity in different manners, some of them become more susceptible and others become more resilient. The prevalence of the metals accumulating in all of samples is an indicator that more than likely these metals are accumulating in other estuarine species as well. If a metal is present in one species, especially in high concentration, then one can assume that other species in the same ecosystem will have accumulated some level of the same metal, resulting in widespread metal toxicity among an ecosystem's community. Aluminum has proven to be quite toxic especially at low pH levels associated with Alum ion concentration. Nickel is known to be a carcinogen and a mutagen much like that of mercury. Ni is able to dissolve and particulate matter, more often to carbon than other organic matter. The bioavailability of Ni can be correlated to the concentrations of calcium and magnesium. Research has shown that Ni is able to cause tissue damage, genotoxicity, growth inhibition in marine ecosystems. This study analyzed a wide range of heavy elements simultaneously within the same sample. The method is non-destructive, hence samples can be stored for future reference or analysis by other laboratories for quality control purposes. This is important in regulatory pollution control work where analytical results can be vital. In addition, this study is sensitive to many elements over a broad span of concentrations and detection range is linear for a large number of elements. This alleviates the necessity for concentrating or diluting samples to within a range suitable for analysis, as required by some other techniques.

Conflict of interest

The authors declare that they have no competing interests.

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